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EXTENDING THE SHELF-LIFE OF A VALUE-ADDED PORK PRODUCT

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EXTENDING THE SHELF-LIFE OF A VALUE-ADDED PORK PRODUCT

Saskatchewan Department of Agriculture & Food

Agriculture Development Fund (ADF)

Project # 20040503

Final Report

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EXECUTIVE SUMMARY

Research was conducted to assess meat factors (pork picnic shoulder and fresh pork sausage patties) and non-meat ingredients (lemon powder, sodium erythorbate) that influence the colour stability of fresh sausage products using a pork patty model system over a typical storage period (5 days). Losses of meat quality were evidenced through the discolouration of meat, depletion of endogenous antioxidant activities, proliferation of spoilage microorganisms, and reduction in the meat redox potential. The quality of both pork picnic shoulder and fresh pork sausage patties decreased with storage at 4°C. Pork picnic shoulder patties, however, had significantly ($p<0.05$) higher activity of catalase, glutathione peroxidise (GSHPx), superoxide dismutase (SOD) and total antioxidant activity (TEAC). The activities of these antioxidant enzymes in both pork picnic shoulder and fresh pork sausage patties were depleted by day 5 of the display period. Moreover, there was no significant treatment effect of the use of ground pork or fresh pork sausage on microbial numbers, but there was a significant ($p<0.05$) elevation of microbial colony forming units by day 5 of the display period. The elevation of microbial numbers by the end of the display period was consistent with the drop in redox potential that was measured near the surface of the patties at the end of the incubation period.

There was no synergistic colour stabilizing effect ($p<0.05$) between sodium erythorbate and lemon juice powder during storage and display. Furthermore, the combination of sodium erythorbate and lemon juice powder did not affect antioxidant activity, colour or the microbiological profile. The addition of sodium erythorbate alone, however, had a significant effect ($p<0.05$) on catalase activity. Losses of meat quality were evidenced through the discolouration of meat, depletion of endogenous antioxidant activities, proliferation of spoilage microorganisms, and reduction in the meat redox potential. Thus, catalase activity was more effective in protecting against oxidation following the addition of sodium erythorbate, resulting in the preservation of redness in the fresh pork sausages (a*value). Notably, the combining sodium erythorbate and lemon juice powder did not significantly affect antimicrobial activity as there was no significant difference in total microbial counts (*Brochothrix thermosphacta* count and lactic acid bacteria) following the addition of those ingredients. The measurement of redox potential near the surface of fresh pork patties could not be conclusively correlated with the addition of non-meat ingredients or microbiological activity. However, redox potential measurements from the middle of fresh pork patties showed that the addition of sodium erythorbate lowered the redox of the fresh pork sausage containing 0.05% sodium erythorbate, as well as sausage containing 0.25% lemon juice powder and 0.05% sodium erythorbate.

TABLE OF CONTENTS

EXECUTIVE SUMMARY 2

Saskatchewan Department of Agriculture & Food 1

Agriculture Development Fund (ADF) 1

Project # 20040503 1

Final Report 1

D.R. Korber and J. Kusuma 1

EXECUTIVE SUMMARY 2

1.0 INTRODUCTION 5

2.0 MATERIALS AND METHODS 7

2.1 STUDY 1: Assessing the stability of fresh pork sausage over time at 4°C 7

2.1.1 Experimental Design 7

2.1.2 Meat processing 8

2.1.3 Colour measurements and haem pigment analyses 10

2.1.3.1 Instrumental colour measurements 10

2.1.3.2 Relative amount of metmyoglobin 10

2.1.4 Redox potential measurements 11

2.1.5. pH determinations 12

2.1.6. Extraction of meat endogenous enzymes 13

2.1.7 Total antioxidant determination 13

2.1.7.1 ABTS•+ preparation 13

2.1.7.2 Trolox standards 13

2.1.7.3 Spectrophotometric assay 14

2.1.8 Superoxide dismutase (SOD) activity measurements 14

2.1.9 Catalase (CAT) activity measurements 15

2.1.10 Glutathione peroxidase (GSHPx) activity measurements 15

2.1.11 Microbial analyses 17

2.1.12 Statistical analyses 17

2.2 STUDY 2 - Assessing the effect of non-meat ingredients on the stability of fresh pork sausage over time at 4°C 18

2.2.1 Experimental Design 18

2.2.2 Meat processing 19

2.2.3 Citric acid determination 21

2.2.4 Statistical analysis 21

3.0 RESULTS AND DISCUSSION 23

3.1 STUDY 1: Assessing the stability of fresh pork sausage over time at 4°C 23

3.1.1 Antioxidant status of meat patties 23

3.1.1.1 The effect of salt on endogeneous antioxidant activities 23
3.1.1.2 Catalase 24
3.1.1.3 SOD 24
3.1.1.4 GSHPx 25
3.1.1.5 Total antioxidant capacity (TEAC) 25
3.1.2 Colour analyses of pork patties 30
3.1.2.1 Relative amount of metmyoglobin 30
3.1.2.2 L* values 31
3.1.2.3 a* values 32
3.1.2.4 b* values 33
3.1.3 Microbial analyses 33
3.1.4 Redox potential analysis 36
3.1.5 Proximate analysis and pH measurement of meat patties 39
3.2. STUDY 2 - Assessing the effect of non-meat ingredients on the stability of fresh pork sausage over time at 4°C 44
3.2.1 Antioxidant activity 44
3.2.2 Colour 44
3.2.3 Microbiology 58
3.2.4 Redox potential 61
3.2.5 Fresh pork sausage using DFD picnic boneless pork 62
3.2.6 The effect of retail light on antioxidant activity, colour and microbial activity 69
4.0 CONCLUSIONS 76
5.0 FUTURE WORK 77
6.0 ACKNOWLEDGEMENTS 78
7.0 REFERENCES 78

1.0 INTRODUCTION

Oxidative processes can result in the deterioration of overall quality of meat products. The colour stability, however, is the most important quality attribute that is closely associated to meat shelf-life; customers relate the bright red colour of oxymyoglobin with meat freshness. Discolouration depends on the overall activities of anti- and pro-oxidants in the meat that either can enhance or inhibit oxidative processes. Therefore, it is important to be able to inhibit or minimize the oxidative processes in meat in order to minimize meat discolouration so that consumer rejection of these products based on the product's visual appearance is reduced.

Meats have endogeneous antioxidant enzymes whose primary function is the protection of cells from oxidation *in vivo* (Gutteridge and Halliwell, 1994). These enzymes are also responsible for meat colour stability *post mortem*, but their effectiveness decreases over time (Monahan, 2000). Important endogenous antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). SOD and CAT are coupled enzymes. SOD catalyzes the conversion of the superoxide anion to hydrogen peroxide wherease CAT catalyzes the conversion of hydrogen peroxide to water and oxygen. The glutathione system (*i.e.*, glutathione, glutathione peroxidase and glutathione reductase) is a key defense system against the build up of H_2O_2 and other peroxides (Gatellier *et al.*, 2004). Meat processing and the addition of non-meat ingredients could affect the stability of these enzymes and thereby affect the stability of meat colour. The estimation of total antioxidant status is more desirable in describing the capacity of the meat to retard the oxidative process because the measurements of each individual antioxidant does not really reflect the overall status of meat antioxidant capacity (Gatellier *et al.*, 2004).

The redox potential of meat is influenced by intrinsic factors related to the product, as well as extrinsic factors imposed during meat processing (Rödel and Scheuer, 2000a,b). The intrinsic factors include the addition of non-meat ingredients and food additives in meat processing; on the other hands, the extrinsic factors include the technical processes. All of these intrinsic and extrinsic factors play a role in defining the quality of the final meat product. Moreover, to predict the overall quality of meat product, it is also important to consider the growth of microorganisms in the meat products, which may, as a consequence of their growth, alter the redox conditions. Thus, the redox potential may function as an indicator of the microbial stability of the meat products, and also be used to assess the process of decomposition of fresh meat during storage to determine the overall meat quality deterioration over time.

The objectives of this study were:

- To determine the stability of pork patties in terms of their chemical (redox potential and antioxidant enzyme activities), colour and microbiological (types and numbers of bacteria) characteristics during storage.

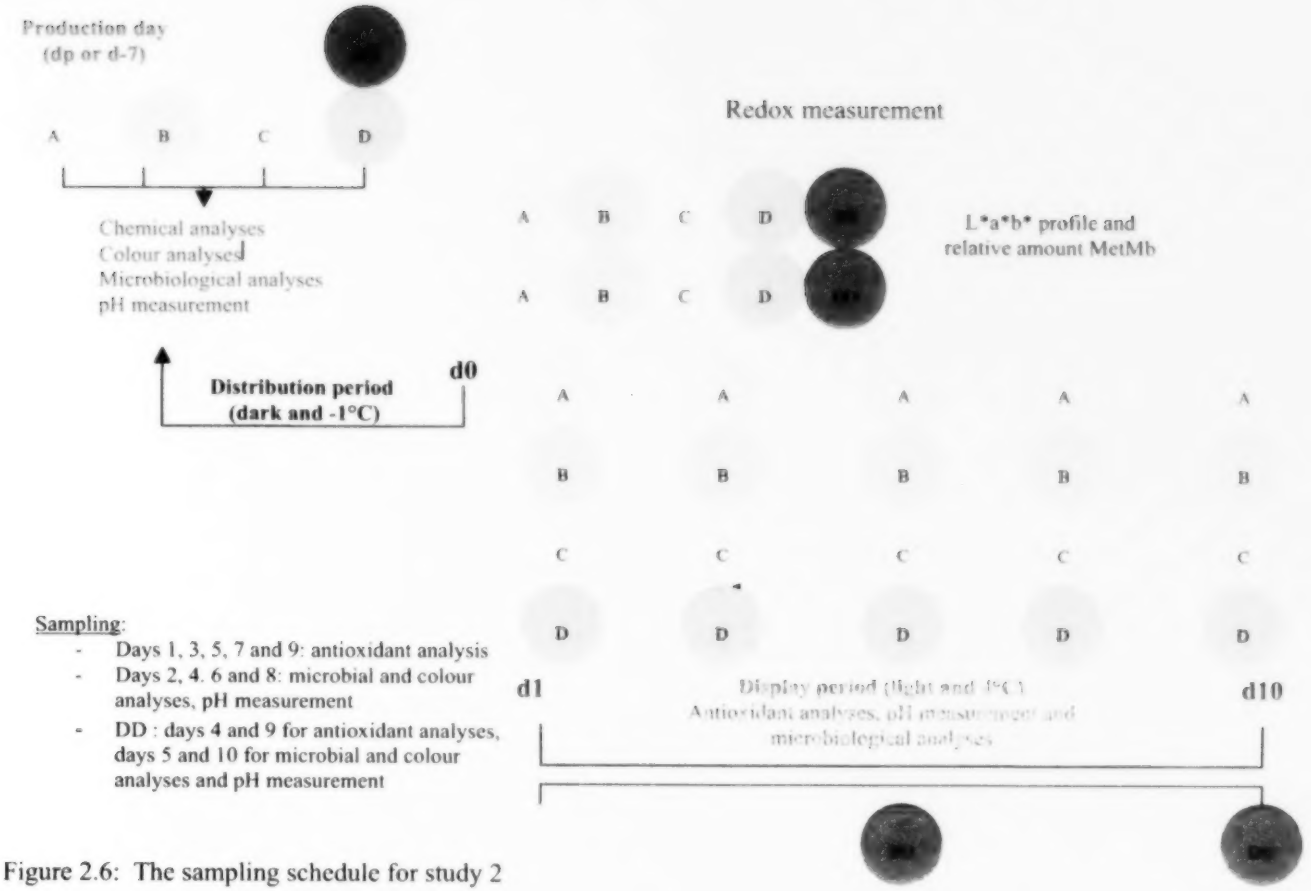
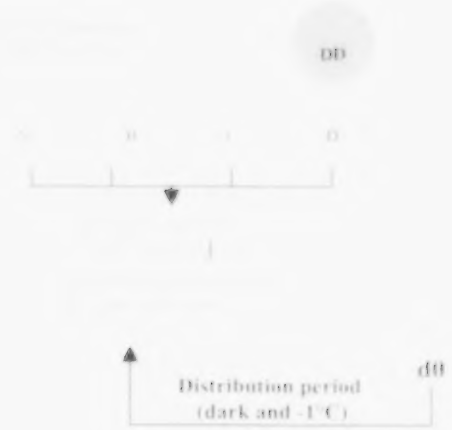


Figure 2.6: The sampling schedule for study 2



Sampling
- Days 1, 3, 5, 7 and 9: antioxidant analysis
- Days 2, 4, 6 and 8: microbial and colour analyses, pH measurement
- DD: days 4 and 9 for antioxidant analyses, days 5 and 10 for microbial and colour analyses and pH measurement

Figure 2.6: The sampling schedule for study 2

- To assess the effect of non-meat ingredients on the stability of pork patties in terms of their chemical (redox potential and antioxidant enzyme activities), colour and microbiological (types and numbers of bacteria) characteristics over time.

2.0 MATERIALS AND METHODS

2.1 STUDY 1: Assessing the stability of fresh pork sausage over time at 4°C

2.1.1 Experimental Design

The main objective of this study was to determine the stability of fresh pork sausages in terms of their chemical, colour, and microbiological characteristics (Figure 2.1) during a typical retail display period. To mimic retail display conditions, fresh pork sausages were placed under fluorescent lights (F40 CW, General Electric, Mississauga, ON, 850-1100 lux) for 5 days at a constant temperature of 4°C. Before the fresh pork sausages were displayed, they were held after production for 7 days in the dark at -1°C, as this represents a typical period before distribution. Besides the fresh pork sausages, ground pork patties were used to assess how the meat itself behaves during the storage and display periods without any addition of ingredients.

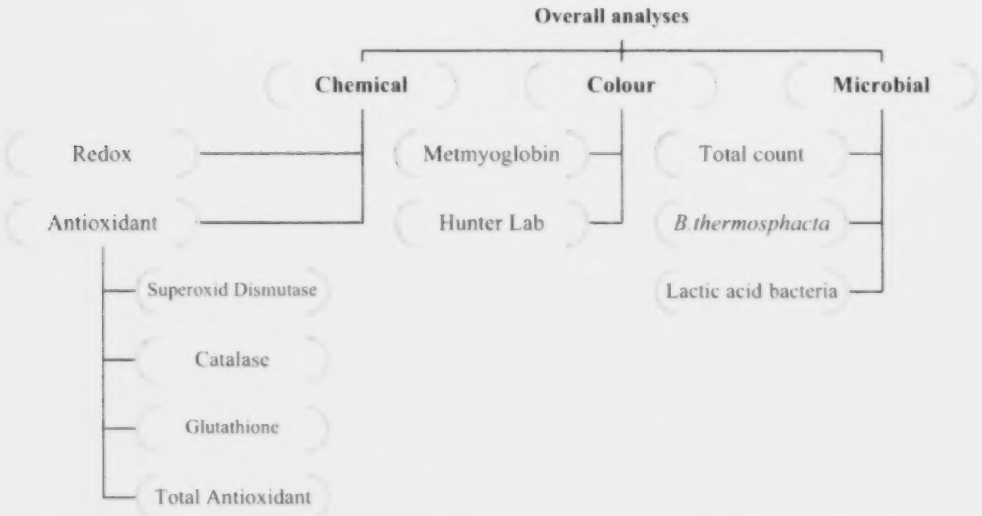


Figure 2.1: The experimental analyses performed on fresh pork sausages in patty form and ground pork patties conducted over days 1 to day 5 of simulated retail display at a constant 4°C and a light intensity of 850 – 1100 lux

Ten patties (P#1 to P#10) for each fresh pork and pork picnic sausage were produced on the production day (day -6). On this day, all analyses, as depicted in Figure 2.1, were performed with the exception of the antioxidant analyses. Patty #1 (P#1) was followed from day 1 to day 5 of the display period for redox measurements. Additionally, duplicate patties (P#2 and P#3) were followed from day 1 to day 5 of the display period for colour measurements. Patties (P#4 to P#8) were sacrificed daily from day 1 to day 5 of the display period for antioxidant and microbiological analyses (Figure 2.2). This schedule was followed for both the fresh pork sausages patties and the ground pork patties. Three batches of sausages were prepared and analyzed to give true experimental replicates.

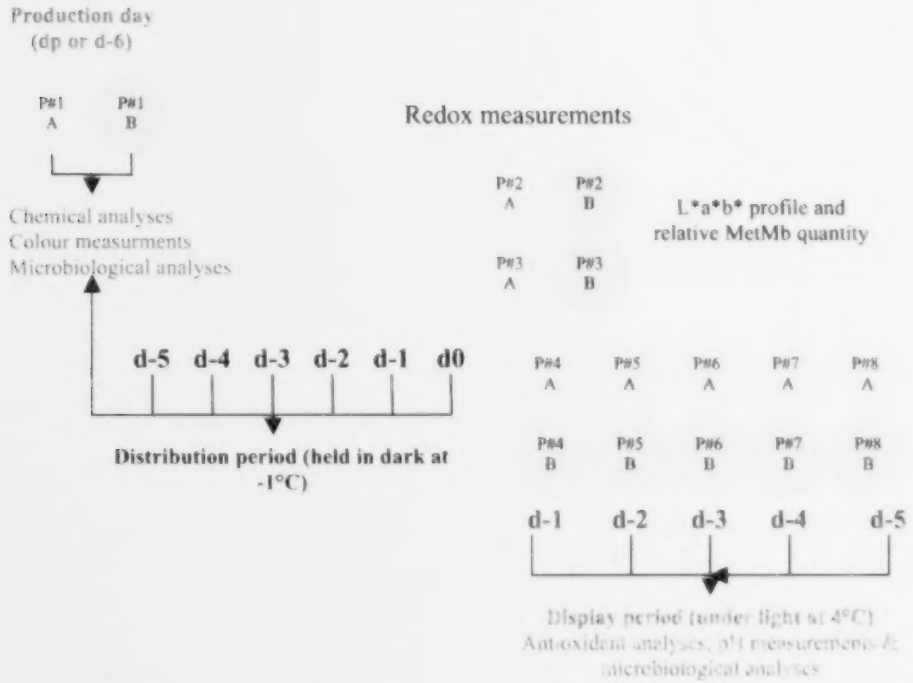


Figure 2.2: The sampling schedule: blue = fresh pork sausages patties; pink = ground pork patties

2.1.2 Meat processing

All meat processing was carried out in the Meat Pilot Plant located within the Department of Food and Bioproduct Sciences in the College of Agriculture & Bioresources at the University of Saskatchewan. A typical fresh pork sausage formulation was used in the preparation of pork patties; targets of 20% (or less) fat content and 14% to 16% protein content in the fresh sausages were specified. The non-meat ingredients included: water/ice (12%, w/w), salt (1.5%, w/w), lemon juice powder (0.25%, w/w), and sodium erythorbate 0.05% (*i.e.*, 500 ppm).

Pork picnic shoulder was obtained from Maple Leaf Foods and used for the formulation of fresh sausage within 10 days of the production date. The composition of the picnic shoulder boneless pork was estimated by using the Pork Picnic Shoulder - USDA Nutrition Database (Table 2.1). The pork picnic (handled in 6 kg batches) was first ground with a Hobart® grinder through an 11/16″ plate (The Hobart, Model 4812, Troy, OH, USA), after which random samples were taken for quick fat analysis (HFT 200, Data Support Co Inc., Encino, CA). A meat mixture with fat content ranging from 16 to 20 % was targeted before proceeding to the subsequent processing steps. After the required amount of fat content was achieved, the meat mixture was then ground twice using a 1/8″ plate. Some sample was taken at this point for proximate analyses (*i.e.*, crude fat, moisture and protein contents). A portion of the ground meat block (~ 3 kg) was then used in the formulation of fresh pork sausages with the specified non-meat ingredients: water/ice (12%, w/w), salt (1.5%, w/w), lemon juice powder (0.25%, w/w), and sodium erythorbate 0.05% (*i.e.*, 500 ppm). The remaining meat was formed into patties. All of the non-meat ingredients were first dissolved in the ice water before they were mixed together with comminuted meat to ensure that the non-meat ingredients were well-mixed. Both meat and non-meat ingredients were combined in a Berkel mixer (Model BA20, Omcan Inc., Mississauga, ON, Table 2.2) for 60 secs before they were portioned using the Omas patty stacker (Model BT10, Omcan Inc., Mississauga, ON) into ~120 g pork patties. All patties were then placed on Styrofoam® trays and over wrapped with a standard oxygen permeable film (AEP Canada Inc., RMF61-HY, Scarborough, ON) with a known oxygen transmission rate of 1400 cm^3/0.06 m^2 per 24 hours. The patties were stored in the dark for 7 days at -1°C (*i.e.*, to simulate a typical distribution scenario) before being placed under fluorescent lighting (850 to 1100 lux) at a constant 4°C for the remaining 5 days display period. Sixteen patties were made in total, with 8 patties prepared for each type (*i.e.*, either pork picnic or fresh pork sausage). Two calibrated thermometers were inserted into Erlenmeyer flasks filled with water held near the pork patties; the temperature was recorded daily over the entire storage period to confirm that a constant storage temperature was maintained.

Table 2.1: The composition of pork picnic shoulder based on the USDA Nutrition Database. Adapted from USDA Nutrition Data Laboratory, 2007

Proximate	**Units**	**Value per 100g**
Water	g	62.06
Energy	kcal	253.0
Energy	kJ	1059
Protein	G	16.69
Total lipid (fat)	G	20.19
Ash	G	0.840
Carbohydrate, by difference	G	0.000
Fibre, Total dietary	G	0.000

Table 2.2: The formulation for fresh sausage patties along with the calculated fat content and protein content

Ingredients	Level (%)	Fat (%)	Protein (%)	Mass (g)
Pork picnic shoulder	86.20	17.40	14.39	2586
Sodium chloride	1.500	0.000	0.000	45.00
Sodium erythorbate	0.050	0.000	0.000	1.500
Lemon juice powder	0.250	0.000	0.000	7.500
Water/ice	12.00	0.000	0.000	360.0
Total	100.0	17.40	14.39	3000

2.1.3 Colour measurements and haem pigment analyses

2.1.3.1 Instrumental colour measurements

Colour measurements (CIE, L^*, a^*, b*) were performed at the surface of the meat samples using a HunterLab colorimeter (MiniScan XE TM Version 3.0 1995) with Illuminant A and 10° standard observer for both pork picnic and fresh pork sausage patties. The colour measurement was taken with the film overwrapping left in place. Standardization of the unit was carried out with white and black tiles that were wrapped with the same over-wrap film as used for the patties (*i.e.*, standard oxygen permeable film, RMF61-HY). A pink tile, with specifications of $L^* = 76.39$, $a^* = 25.57$ and $b^* = 17.75$, was read to confirm that the unit was functioning properly before colour measurements of the meat patties were taken. Two patties for both pork picnic and fresh pork sausage patties were measured every day; 2 measurements (*i.e.*, at 90° rotation to one another) were recorded from each patty so that an average from 4 measurements could be reported.

2.1.3.2 Relative amount of metmyoglobin

The HunterLab MiniScab XE TM was also employed to determine the percent metmyoglobin at the surface of the meat patties. Accordingly, reflectance was measured in the form of K/S (Kubelka – Munk) values between 400 nm and 700 nm at 10 nm intervals. The relative content of metmyoglobin was estimated by calculating the ratio of $K/S_{572/525}$ as described by Hunt *et al.* (1991). K/S ratios at 572 nm and 525 nm, which were not given by the instrument, were calculated using linear interpolation. This analysis was conducted to monitor changes of the relative amount of metmyoglobin on a day-to-day basis during storage, so that K/S conversion was not necessary. As the metmyoglobin content increases with storage, K/S ratios decrease correspondingly, so the K/S ratios were multiplied by -1 so that K/S ratios increase in the diagram (Lindahl *et al.*, 2006a). Two patties for both pork picnic and fresh pork sausage patties were measured every day; two measurements (*i.e.*, at 90° rotation to one another) were recorded from each patty so that an average from 4 measurements could be reported.

2.1.4 Redox potential measurements

The measurement of meat patty redox potential was performed by using microelectrodes that were connected to a data logger (51x Micrologger, Campbell Scientific Inc., Edmonton, AB). The redox potential of fresh pork sausage patties and ground pork patties were measured over time. Two microelectrodes and 1 reference electrode were inserted into each patty with 1 microelectrode located near the patty's surface and other microelectrode was place into the middle portion of the patty (Figure 2.3).

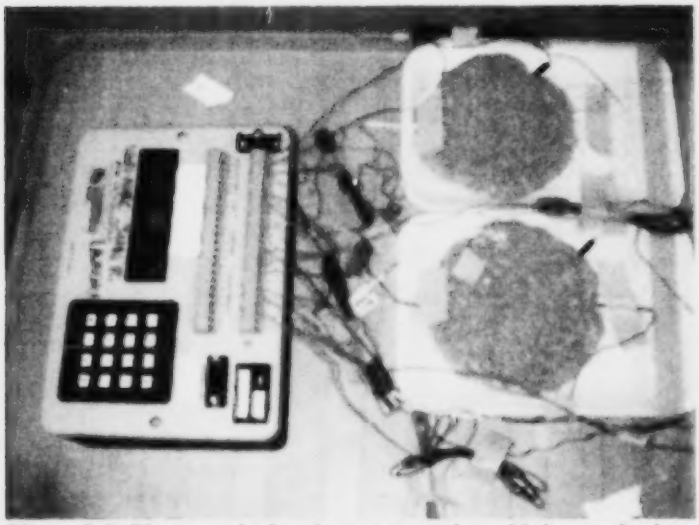


Figure 2.3: Photograph showing meat patties with inserted microelectrodes wired to the data logger. Each patty had one microelectrode inserted near the surface of the patty, a second microelectrode inserted near the middle of the patty and a reference electrode in the middle of the patty.

Redox potential values were determined using a AgCl microelectrode as a reference; the conversion of E to E_h (*i.e.*, standard hydrogen electrode as reference electrode, Equation 3.1) was not necessary because the trend of the redox potential values during both the first 7 days of storage at -1°C in the dark, and 5 days of storage at 4°C in the light, was more important than the exact values.

$$E_h = E + E_{ref} \qquad \text{(Equation 2.1)}$$

E_h = potential (mV) of sample solution relative to the standard hydrogen electrode.

E = potential (mV) of the sample measured at a specified temperature.

E_{ref} = reference electrode potential. For Ag:AgCl – saturated KCl a value of +199 ± 5 mV was used.

The efficacy of the microelectrode pair was checked in Zobell solution prior to the analyses in order to confirm that the microelectrodes were functioning properly. The Zobell solution consists of a 0.1 molal KCl solution containing equimolal amounts of $K_4Fe(CN)_6$ (potassium ferrocyanide) and $K_3Fe(CN)_6$ (potassium ferricyanide). The solution was prepared by dissolving 1.4080 g **$K_4Fe(CN)_6 \cdot 3H_2O$**, 1.**0975g $K_3Fe(CN)_6$**, and **7.4557 g KCl** in deionized water and then diluting to 1 L. The E_h value of the Zobell solution is temperature-dependent (Table 2.3); thus, the E_h reading at room temperature should be 430 mV. The redox potential measurements were performed in the same cold room where the patties were stored, so that both light intensity and temperature could be held constant for all patties during the entire storage period.

Table 2.3: Eh of Zobell's solution as a function of temperature

Temperature °C	E_h (mV)
10	467
12	462
14	457
16	453
18	448
20	443
22	438
24	433
25	430
26	428
28	423
30	418
32	416
34	407
36	402
38	397
40	393

Adapted from Nordstrom and Wilde, 1998

2.1.5. pH determinations

Slurry pH measurements were conducted in duplicate every day from day 1 to day 5 for both pork picnic and fresh pork sausage patties. For each patty, 20 g of sample was blended with 80 mL distilled water and the pH values determined within 1 mins following blending. The pH meter (Fisher Scientific, Accumet AB15 plus Brinkmann Instrument Canada, Nepean, ON) was calibrated with pH 4.0 and pH 7.0 buffer solutions before the pH measurements were made. The measurements were performed in duplicate.

2.1.6. Extraction of meat endogenous enzymes

A 5 g sample of meat was aseptically taken from the surface of each designated patty and transferred to a 50 mL polypropylene Falcon® centrifuge tube. The tube was immersed in a beaker filled with ice water, and its contents were homogenized with 20 mL of 50 mM potassium buffer (pH 7.4) using a Polytron homogenizer (PT10135, Brinkmann Instrument Canada, Mississauga, Ontario) for 20 secs at 9000 rpm. The sample was then centrifuged (Beckman, model J2HC, rotor JA17, Beckman Coulter, Mississauga, ON) at 9000 rpm for 20 mins at 4°C. The resultant supernatant was filtered through filter paper (Whatman no 4.25 µm particle retention, VWR Canlab, Mississauga, ON) into a graduated cylinder so that the volume of the extract could be recorded before its transfer to a 25 mL Erlenmeyer flask. This extract was used as a working solution for all antioxidant analyses. The volume of the working solution after extraction was measured for final calculation of enzyme activity.

2.1.7 Total antioxidant determination

The Trolox Equivalent Antioxidant Capacity (TEAC) procedure was used to measure the total antioxidant activity of meat extract, as described by Re *et al.* (1999). This method is based on the relative capacity of hydrogen-donating antioxidants to scavenge the pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) [$ABTS^{\bullet+}$] compared to the antioxidant potency of 2.0 mM Trolox as a standard. One unit activity of TEAC is defined as the concentration (mmol/L) of Trolox having the equivalent antioxidant capacity of a 1.0 mmol/L solution of the substance under investigation (Miller *et al.*, 1993). All the chemicals used in this analysis were obtained from Sigma Aldrich (Oakville, Ontario).

2.1.7.1 $ABTS^{\bullet+}$ preparation

The oxidation of 7 mM ABTS to $ABTS^{\bullet+}$ with 2.45 mM potassium persulfate was carried out by mixing 385 mg ABTS and 66 mg $K_2S_2O_8$ with 100 mL of 5 mM PBS (pH 7.4) in Erlenmeyer flasks. After 12-16 hours incubation, the $ABTS^{\bullet+}$ stock solution was filtered using filter paper (Whatman no 4, 25 µm particle retention, VWR Canlab, Mississauga, ON). This stock solution was then diluted with 5 mM PBS (pH 7.4) until an absorbance reading of 0.70 (± 0.02) at 734 nm was obtained. Note that the final absorbance reading of 0.70 (± 0.02) at 734 nm was made after the diluted $ABTS^{\bullet+}$ solution had been equilibrated at 30°C. The spectrophotometer (Agilent 8453 UV Visible, Agilent Technology, Mississauga, ON) was first zeroed with 5 mM PBS (pH 7.4) before sample readings were conducted.

2.1.7.2 Trolox standards

A 2 mM Trolox solution was prepared by weighing 50 mg 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid into 100 mL of 5 mM PBS (pH 7.4). Serial dilutions were then performed to obtain Trolox working solutions with final concentrations of 2, 8, 12, 16, and 20 µM. The Trolox standard then was used to determine the activity unit of TEAC of the sample (Figure 2.4).

2.1.7.3 Spectrophotometric assay

After the addition of 10 µL Trolox standard or meat sample extract (1:2) to 1 mL of the $ABTS^{\bullet+}$ solution, the absorbance at 734 nm was measured using a spectrophotometer at 30°C at exactly 6 mins after initial mixing. The percent inhibition was then calculated as shown in Equation 3.2. A standard curve depicting % inhibition *vs.* concentration was constructed. The absorbances of the standards were read in duplicate for each concentration. The absorbances of the pork sample extracts were similarly measured in triplicate for both pork picnic and fresh pork sausage patties.

$$\% \text{ Inhibition} = \frac{Abs_{734} \text{ (blank – standard or sample) at 6 min}}{Abs_{734} \text{ (blank) at 6 min}} \times 100\%$$

(Equation 2.2)

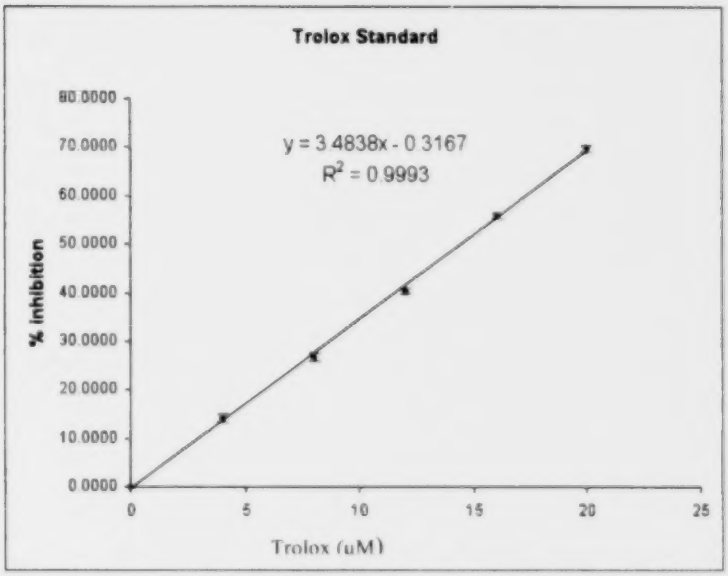


Figure 2.4: Trolox standard curve for the determination of TEAC value

2.1.8 Superoxide dismutase (SOD) activity measurements

The activity of SOD was measured using the method of Markulund and Markulund (1974). This method involves the inhibition of pyrogallol autooxidation in basic medium. The rate of pyrogallol autooxidation in the sample extract was then compared with the blank (*i.e.*, the phosphate extraction buffer) by measuring the increase in absorbance at 340 nm during the first 2 mins (in duplicate) at room temperature (Gatellier *et al.*, 2004). One unit of SOD is defined as the activity of sample that inhibits the reaction by *50%* (Renerre *et al.*, 1996; Gatellier *et al.*, 2004). All the chemicals used in this analysis were obtained from Sigma Aldrich.

In this study, the sample standard was constructed by measuring the sample extract at different volumes (Table 2.4) so that % inhibition at different sample extract concentration can be obtained. From this curve (volume of sample extracts vs. % inhibition), a straight line equation can be derived. From this equation, the volume of the meat extract that has 50% inhibition can be determined as one unit.

The % inhibition was then calculated (Equation 2.3). The undiluted meat extract must have a % inhibition of greater than 50%; this was achieved by adjusting the volume of meat extract and buffer in the system. In the case where a 50 µL meat extract sample with 0 µL added buffer demonstrated a % inhibition less than 50%, then the quantity of meat extract was increased to either 60 or 70 µL. In the sample standard, 5 points, including 0% inhibition in 0 µL meat extract sample with 50 µL buffer, were used to obtain the curve. The final SOD activity was expressed as IU/ g of meat. The spectrophotometer was zeroed with 50 mM Tris-HCl buffer (pH 8.2) prior to reading the samples.

$$\% \text{ inhibition} = \frac{(\text{slope of blank} - \text{slope of meat extract})}{\text{slope of blank}} \times 100\% \qquad \text{(equation 2.3)}$$

2.1.9 Catalase (CAT) activity measurements

Catalase activity was determined by the method of Aebi (1983). Briefly, 1.0 mL of meat extract (diluted 1:8 with 50 mM phosphate buffer pH 7.4), 1.5 mL 50 mM phosphate buffer (pH 7.4) and 0.5 mL 100 mM H_2O_2 were added to a quartz cuvette. The activity of catalase in the meat extract was then determined in duplicate by monitoring the decrease in absorbance at 240 nm at room temperature during the first 30 secs for 1 sec interval. One unit of catalase activity is defined as the quantity of meat extract needed to decompose 1 µmol of H_2O_2/min (Hernández *et al.*, 2002). The extinction coefficient of H_2O_2 (43.6/M cm) was used for the calculation of catalase activity (U/g of meat). The spectrophotometer was first zeroed with 2.0 mL 50 mM phosphate buffer (pH 7.4) and 1.0 mL meat extract (diluted 1:8 with 50 mM phosphate buffer pH 7.4) prior to reading because the contribution of the haem meat pigments needed to be subtracted from the overall absorbance reading before the measurement of the catalase activity. All the chemicals used in this analysis were obtained from Sigma Aldrich.

2.1.10 Glutathione peroxidase (GSHPx) activity measurements

The enzymatic activity of GSHPx was measured using the indirect, coupled test procedure as described by Agergarrd and Thode Jansen (1982). As shown in Figure 2.5, the glutathione redox cycle is a central mechanism for the reduction of intracellular hydroperoxides by reducing H_2O_2 to H_2O via the oxidation of glutathione (GSH). The oxidized glutathione (GSSG) is reduced back to GSH by the enzyme GSH reductase (GR), a reaction requiring NADPH regenerated by glucose 6-phosphate dehydrogenase (G6PDH). All the chemicals used in this analysis were obtained from Sigma Aldrich. GSHPx activity was measured with GSH reduction coupled to NADPH oxidation by the enzyme gluthathione reductase. The sample extract was added to the reaction medium (Table 2.5) and the rate of NADPH oxidation at 37°C measured at 340 nm in duplicate

over 5 mins. This reading was taken 1 mins after the reaction was initiated via the addition of tert-butyl hydroperoxide, TBHP. GSHPx activity is the amount of extract that is required to oxidize 1 μmole NADPH/min at 37°C (Hernandez et al., 2004). The extinction coefficient of NADPH is 6330/M cm. The spectrophotometer was zeroed with a 16 mM EDTA in 400 mM phosphate buffer (pH 7.4) prior to sample measurements.

Table 2.4: Meat extract dilution to establish sample standard

Meat extract (μL)	50 mM phosphate buffer pH 7.4 (μL)
50	0
40	10
30	20
20	30
10	40

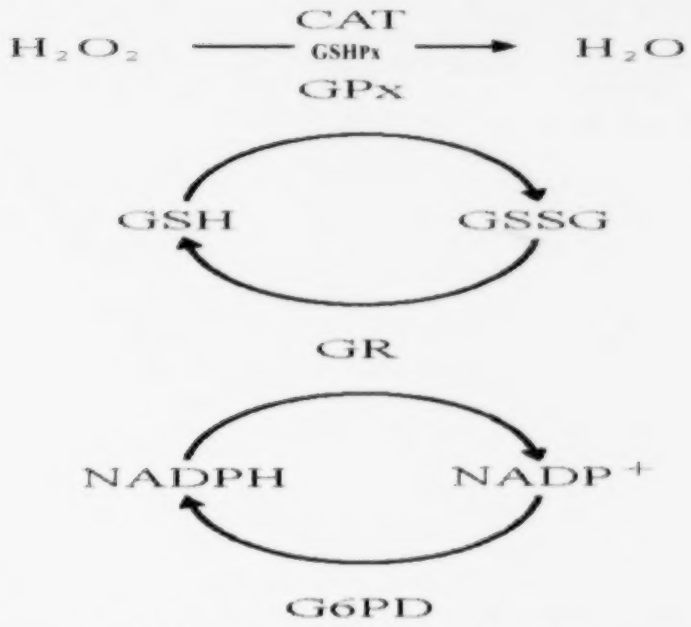


Figure 2.5: Glutathione redox cycle. Adapted from Heffner and Repine, (1989) GSHPx = Glutathione peroxidase, GSH = Glutathione, GSSG Oxidized glutathione, GR = Glutathione reductase, NADP = Nicotinamide adenine dinucleotide phosphate, NADPH = Reduced nicotinamide adenine dinucleotide phosphate, G6PD = Glucose 6-phophate dehydrogenase

Table 2.5: The reaction medium in the cuvette for GSHPx determinations

	Volume (µL)
16 mM EDTA in 400 mM phosphate buffer (pH 7.4)	500
Glutathione reductase 15 U/mL	50
60 mM Glutathione reduced (GSH)	200
50 mM phosphate buffer (pH 7.4) or meat extract	100
Deionized water	400
Incubation for 10 min at 37°C	
3 mM NADPH in 0.1% (w/w) $NaHCO_3$	200
6.3 mM *tert*-butyl hydroperoxide (TBHP)	500

2.1.11 Microbial analyses

Ten gram samples of pork patties that had been aseptically scraped from the surface of the patties were homogenized in a Stomacher blender (Stomacher® LabBlender 400, St. Edmunds) with Seward BA6041/STR filter bag (VWR, Edmonton, AB) in 90 mL of sterile 0.1% (w/v) peptone water for 1 mins. The homogenate was serially diluted (4 times) using 0.1% sterile peptone water before plating. Three plates were prepared for each dilution by spreading 0.1 mL of each dilution on agar plates. Three types of media were used to assess the microbiological flora present on both fresh pork sausages and pork picnic patties:

- Trypticase Soy Agar (TSA;BBL, Becton Dickinson, Cockeysville, MD) + 0.1% (w/v) yeast extract (Difco Laboratories, Detroit, MI), incubated for 24 hours at 37°C for the isolation and cultivation of a wide variety of heterotrophic microorganisms;
- De Man Ragosa and Sharpe Agar (MRS; EMD Chemicals, Darmstadt, Germany) for 72 hours at 30°C for the cultivation of lactic acid bacteria; and
- Streptomycin Thallous Acetate Actidione (STAA; Oxoid, Nepean, ON) Agar incubated for 48 hours at 25°C aerobically for the cultivation of *Brochothrix thermosphacta* (Gardner, 1985).

Microbial enumeration was performed using the plate count method (spread plate) in triplicate and was presented as the number of colony forming units (CFU) per gram of meat.

2.1.12 Statistical analyses

The colour, chemical, and microbiological data were analysed as repeated measures with a Completely Randomized Design using the Mixed Model procedure of SAS (SAS Institute Inc., 1999). The mixed procedure was applied when calculating the least squares means (LSM) and standard error (SEM). Both treatments (*i.e.*, fresh pork sausages and ground pork) were analyzed in triplicate (3 batches) from day 1 to day 5, for each study. The Satterthwaite's approximation on degrees of freedom was used with the Kenward-Roger adjustment on standard errors. The pdiff test was used to determine the least significance difference between LSM. Significance was declared at $p<0.05$, or

as otherwise indicated. Correlation coefficients among colour, chemical, and microbiological analyses data were also performed using the Paerson's correlation of SAS (SAS Institute Inc., 1999).

2.2 STUDY 2 - Assessing the effect of non-meat ingredients on the stability of fresh pork sausage over time at 4°C

2.2.1 Experimental Design

The objective of this study was to assess the effect of non-meat ingredients, which include lemon juice powder and sodium erythorbate, on the stability of fresh pork sausages in terms of their chemical, colour, and microbiological characteristics over time at a constant temperature of 4°C under fluorescent lighting of 850 -1100 lux. The lemon juice powder was obtained from Newly Weds Foods Co. (Mississauga, ON). It was comprised of lemon juice solids and maltodextrin, and had moisture content less than 5.5%. Nutritional facts of the lemon juice powder from Newly Weds Food Co (Mississauga, ON) are provided in Table 2.6. The citric acid content in lemon juice powder was measured by an acid titration method as described below.

The sampling schedule is described in Figure 2.6. On the production day, 8 fresh pork sausage patties were made for each type of fresh sausage A, B and C. Twelve fresh pork "D" sausages were made as 4 extra fresh pork sausages D were needed for the light analyses. During the display period, fresh pork sausages were displayed under the fluorescent light (General Electric F40 CW, 850-1100 lux) at constant 4°C for 10 days. Before the fresh pork sausages were displayed, they were held after production for 7 days in the dark at -1°C, representing a typical distribution time. The antioxidant analyses were performed on odd days (day 1, 3, 5, 7 and 9). Two thermometers were inserted into water in the Erlenmeyer flasks near the pork patties and the temperature was recorded daily over the whole storage period to confirm that temperature was held constant during storage. The microbial analyses, colour analyses and pH measurement were performed on even days (day 2, 4, 6, 8 and 10). The effect of light was assessed by displaying 4 fresh pork sausages with no light at a constant 4°C for ten days. Two patties were kept for 10 days for colour analyses which were performed on days 6 and 10. Microbial analyses and pH measurements were also performed on days 6 and 10. Antioxidant analyses were performed on days 5 and 9.

Table 2.6: Nutrition facts per 100 g of lemon juice powder. Powder was obtained from Newly Weds Food Co

Nutrition	Value
Energy	397calories
Fat	0.00g
Saturated	0.00g
Trans	0.00 g
Cholesterol	0.00 g

Sodium	107 mg
Carbohydrate	91.2 g
Fiber	0.00 g
Sugars	5.00 g
Vitamin A	0.00g
Vitamin C	0.00 g
Calcium	1.00 mg
Iron	0.00 g

In order to determine the effect of lemon juice powder and sodium erythorbate on the chemical, colour, and microbiological stability of 4 types of fresh pork sausage (Table 2.7), the proportion of all meat and non-meat ingredients were maintained as given in Study# 1; these ingredients were kept constant in each type of the fresh pork sausage. The meat ingredients, water/ice and salt were the same in all sausages, with lemon juice powder and sodium erythorbate being the variable ingredients.

All chemical, colour, and microbiological analyses were performed as previously described with the exception of total antioxidant analysis (TEAC). Study# 1 revealed that the TEAC analysis did not give very much useful information regarding the antioxidant status of the samples unless other total antioxidant analysis, such as FRAP analysis, was performed along with TEAC analysis. Each type of fresh sausage was sampled as described previously (see Figure 2.1). Replication was also derived from 3 different batches of pork picnic meat for fresh pork production.

2.2.2 Meat processing

The production of fresh pork sausages was performed according to the same protocol as in Section 3.1.2 with the exception of the mixing step. Smaller portions of meat and non-meat ingredients, as indicated in Table 2.8, were blended in the mixer (Kitchenaid ®, model K45, Hobart MTG. Co.,Troy, ON) for 20 s at the lowest speed prior to formation of fresh sausages Type A, B, C, and D in the form of patties.

Table 2.7: Type of sausages examined during this study

		Sodium erythorbate	
		0%	0.05%
		Type A	Type B
Lemon juice powder	0%	(0%, 0%) Type C	(0%, 0.05%) Type D
	0.25%	(0.25%, 0%)	(0.25%, 0.05%)

2.2.3 Citric acid determination

The content of citric acid in the lemon juice powder was determined by a simple acid titration. It is important to note that citric acid ($H_3C_6H_5O_7$) contains 3 carboxylic acid functional groups. When dissolved in water, the molecule exists as a triprotic acid capable of donating 3 protons. Each proton (H^+) from the carboxylic acid group will yield a molecule of water when citric acid reacts with strong base (^-OH).

A 2% (w/v) lemon juice powder solution was prepared by weighing 2 g of lemon juice powder into a 100 mL volumetric flask and then filling it with deionized water. The pH was measured before titration with a standardized 0.1 N NaOH solution. Five mL of the lemon juice solution was quantitatively pipetted into a 250 mL Erlenmeyer flask. Then, ~30 mL deionized water and 3 drops of a 0.1% (w/w) phenolphthalein indicator prepared in 95% (v/v) ethanol were added to the flask. The volume of NaOH required to neutralize the lemon juice solution was recorded. The number of moles of citric acid in the lemon juice solution was calculated and the % (w/w) citric acid determined.

2.2.4 Statistical analysis

The results for colour, chemical, and microbiological data were analyzed as repeated measures with a factorial treatment design in a completely randomized design using the Mixed Model procedure of SAS (SAS Institute Inc., 1999). The 2 x 2 factorial treatment design was used with 2 levels of sodium erythorbate (0% and 0.05%) as one factor and 2 levels of lemon juice powder (0% and 0.25%) as the other factor. The mixed procedure was applied when calculating the least squares means (LSM) and standard error (SEM). All different 4 type of fresh pork sausages were analyzed in 3 batches from day 1 to day 7. The Satterthwaite's approximation on degrees of freedom was used with the Kenward-Roger adjustment on standard errors. The pdiff test was used for least

significance difference between LSM. Significance was declared at $p<0.05$, or as otherwise indicated.

Table 2.8: The formulation for Type A, Type B, Type C, and Type D with the calculated fat content and protein content

Ingredients	Level (%)	Fat (%)	Protein (%)	Weight (g)
Type A				
Pork picnic shoulder	86.20	17.40	14.39	1293
Salt	1.500	0.000	0.000	22.50
Sodium erythorbate	0.000	0.000	0.000	0.000
Lemon juice powder	0.000	0.000	0.000	0.000
water	12.00	0.000	0.000	180.0
Total	**99.70**	**17.40**	**14.39**	**1495**
Type B				
Pork picnic shoulder	86.20	17.40	14.39	1293
Salt	1.500	0.000	0.000	22.50
Sodium erythorbate	0.000	0.000	0.000	0.000
Lemon juice powder	0.250	0.000	0.000	3.750
water	12.00	0.000	0.000	180.0
Total	**99.95**	**17.40**	**14.39**	**1499**
Type C				
Pork picnic shoulder	86.20	17.40	14.39	1293.00
Salt	1.500	0.000	0.000	22.50
Sodium erythorbate	0.050	0.000	0.000	0.750
Lemon juice powder	0.000	0.000	0.000	0.000
water	12.00	0.000	0.000	180.0
Total	**99.75**	**17.40**	**14.39**	**1496**
Type D				
Pork picnic shoulder	86.20	17.40	14.39	1293'
Salt	1.500	0.000	0.000	22.50
Sodium erythorbate	0.050	0.000	0.000	0.750
Lemon juice powder	0.250	0.000	0.000	3.750
water	12.00	0.000	0.000	180.0
Total	**100.0**	**17.40**	**14.39**	**1500**

3.0 RESULTS AND DISCUSSION

3.1 STUDY 1: Assessing the stability of fresh pork sausage over time at 4°C

3.1.1 Antioxidant status of meat patties

Muscle foods are susceptible to oxidative deterioration due to combination of lipid catalyst and membrane lipid systems that are high in unsaturated fatty acids. In general, undesired lipid oxidation could lead to alterations in flavour, odour and colour in meat products. This process of oxidation is enhanced under certain conditions such as grinding (which incorporates oxygen into muscle) and cooking (that releases protein-bound iron into the intracellular pool). The ultimate lipid oxidation potential of meat and meat products is dictated by the balance between prooxidants and antioxidants. Fresh pork has a limited shelf and storage life, even during frozen storage, relative to fresh beef, because pork in general contains more polyunsaturated fatty acids that are more susceptible to lipid oxidation (Benedict *et al.*, 1975).

3.1.1.1 The effect of salt on endogeneous antioxidant activities

It is known that the oxidative stability of muscle foods can be altered by food processing operations and food additives. NaCl is known to have prooxidant traits over the concentration range of 0.5% - 2.0% (Rhee *et al.*, 1983) by compromising the ability of endogenous antioxidant enzymes since, in general, enzyme activities are affected by electrolytes and ionic strength (Richardson and Hyslop, 1985). NaCl may alter the reactivity of iron by the sodium ion (Kanner *et al.*, 1991), activation of the chloride ion via myeloperoxidase (Kanner and Kinsella, 1983), and salt-induced changes in cellular organization (Shommer *et al.*, 1987).

Overall, the catalase, SOD, GSHPx and total antioxidant capacity activities were significantly higher in fresh pork sausages patties than in ground pork patties (Table 3.1 and 3.2). Salt may be responsible for this effect because salt has a prooxidant effect. This prooxidant activity can accelerate lipid oxidation (Tan and Shelef, 2002) and also limit the endogenous enzyme activities (Hernández *et al.*, 2002). Indeed, Lee *et al.* (1997) concluded that it compromised the antioxidant activity in muscle by decreasing the catalytic activity of endogenous antioxidant enzymes.

3.1.1.2 Catalase

The catalase activity had an overall decreasing trend as seen in Table 3.3; however, the drop in catalase activity was more vigorous over day 1 to day 3 than from day 3 to day 5 for both ground pork and fresh pork sausage patties. This suggests that the depletion rate of catalase activity was higher at the beginning than at the end of the storage period. Lee *et al.* (1997) showed that catalase activities in ground pork boston butts (< 3 days *post mortem*) which was stored at -15°C decreased during the first week of storage, but became more stable thereafter. Renerre *et al.* (1996) also indicated that the catalase activity in beef that was stored 2°C wrapped in oxygen permeable film became more stable after 8 days of storage at 2°C. On the other hand, Pradhan *et al.* (2000) showed that the activity of catalase did not change notably in ground pork Boston butt lean over 4 days of storage at 4°C, with an activity of 1144 U/g on day 0, 1092 U/g on day 2 and 1048 U/g for day 4. These values are much higher than the catalase activity seen in either ground pork and fresh pork sausage patties examined this study (Table 3.3). Biological variation or different type of meat could also cause these observed differences.

Pradhan *et al.* (2000) reported that antioxidative processes during storage and distribution of raw meat products is due to catalase activity, so its activity could potentially delay the oxidative process in stored meats. If catalase inhibits oxidation processes, it could in turn protect meat colour from discolouration since lipid oxidation can lead to meat dicolouration. The redness of the patty is the most important criteria for the consumer at the point of purchasing, and this redness is associated with a^* values. Catalase activity had significant positive correlation with a* in both ground pork (0.66) and fresh pork sausage (0.80) patties (Table 3.4 and 3.5). This could suggest that catalase could have a correlation in preserving meat colour in this study.

3.1.1.3 SOD

In this study, SOD decreased more rapidly from day 3 to day 5 than from day 1 to day 3; this trend was more obvious in fresh pork sausage patties than in ground pork patties (Table 3.1). In other words, the activity of SOD was stable in the beginning of the display period but then started to decrease towards the end of the display period. This could suggest that SOD may be more efficient as an antioxidant during the early stages of display. Therefore, even though SOD and catalase are coupled enzymes and thus would be expected to behave in a similar fashion, the evolution of the two enzyme's activities was not the same *post mortem* and there is a possibility that catalase present in meat protects SOD against inhibition of hydrogen peroxide at the beginning of the display period. Despite this difference in trend, both of the activities of SOD and catalase help to scavenge the highly-reactive superoxide and hydroxyl ion, respectively, so that any decrease in their activity may due to oxidative stress *in vivo* (Pigeolot *et al.*, 1990). Anton (1993) also noted that the radical attack was almost completely inhibited when both SOD and catalase were added together. Table 3.5 shows that there was a significant correlation between SOD activity and a^* values. Again, this suggests that there was a significant relationship between SOD and the redness for both fresh sausages and ground pork (Table 3.4 and 3.5).

3.1.1.4 GSHPx

In Table 3.1, the only significant difference observed was for treatment. There was no day effect in this study, even though the activity of GSHPx was higher in ground pork than in fresh pork sausage (Table 3.3). This antioxidant enzyme is relatively stable in meat during refrigerated storage, and in beef has been proposed to offer protection against free radicals *post mortem* (Hernandez *et al.*, 2002). This is supported by Renerre *et al.* (1996) who stated that GSHPx activity did not significantly differ among different beef muscles and with time. However, these results contradict Hernandez *et al.* (2002), who observed that GSHPx activity decreased in both red and white muscles during storage at 4°C for 0, 2 and 4 days (wrapped with polyvinyl chloride film). Lee *et al.* (1997) also showed a similar decreasing pattern of GSHPx activity in pork muscles after 10 week storage at -15°.

3.1.1.5 Total antioxidant capacity (TEAC)

The total antioxidant capacity for both ground pork and fresh pork sausge was decreased significantly during the display period (Table 3.1).Renerre *et al.* (1996) stated the increase of free iron in beef during storage may induce protein oxidation which can lead to the destruction of antioxidant enzymes so they can not perform their function as free-radical scavengers as the storage period increases. Their result clearly reflects the findings of this study, where the TEAC decreased over time. Oxidative stress can lead to meat dicolouration by the oxidation of both deoxymyoglobin and oxymyoglobin to metmyoglobin, so the reduction of TEAC in both ground pork and fresh pork sausage patties could be associated with meat discolouration. However, the results from this study show that there was no significant relationship between total antioxidant capacity with a* redness (Table 3.4 and 3.5). Therefore, the total antioxidant capacity of both the ground pork and fresh pork sausage patties could not be correlated with the colour stability as in the case for catalase and SOD.

It is important to note that TEAC only measures the radical scavenging capacity of meat by inhibiting the autoxidation of $ABTS^{\cdot}$ and thus does not indicate the reducing potential of the sample. Each antioxidant has its own mechanism in inhibiting the oxidation process (Gatellier *et al.*, 2004). Accordingly, an estimation of global antioxidant status rather than single measurement of antioxidant capacity should be used to assess the capacity of muscle to resist oxidation. Therefore, multiple methods must be used to get a better idea of antioxidant status in biological systems.

Table 3.1 The effect of display period (time) and treatment (ground prok and fresh pork sausage) on antioxidant capacity, colour and microbial count analyses during a five day display period at constant 4°C (three batches)

Variables	Treatment	Display time					SEM	*p* values		
		day 1	day 2	day 3	day 4	day 5		trt	day	trt*day
Catalase activity (U/g meat)	Ground pork	295	243	174	160	105	3.323	0.0001	<0.0001	0.1397
	Fresh pork sausage	276	202	134	120	87.1				
GSHx activity (U/g meat)	Ground pork	0.718	0.862	0.787	0.822	0.936	0.2466	0.0023	0.3161	0.1255
	Fresh pork sausage	0.735	0.700	0.612	0.655	0.699				
SOD activity (IU/g meat)	Ground pork	50.6	49.0	46.0	44.7	39.1	1.594	0.0155	<0.0001	0.6526
	Fresh pork sausage	45.0	39.7	40.0	35.7	29.0				
TEAC (mmole Trolox/L)	Ground pork	0.018	0.017	0.016	0.015	0.014	0.0195	0.0010	<0.0001	0.9243
	Fresh pork sausage	0.016	0.015	0.014	0.013	0.012				
Relative amount of Metmyoglobin	Ground pork	1.01	1.04	1.02	1.00	0.870	0.2904	0.0590	<0.0001	<0.0001
	Fresh pork sausage	1.13	1.06	1.03	0.993	1.04				
L* values	Ground pork	58.9	58.5	58.6	58.6	58.4	0.6386	0.5649	0.0001	0.5717
	Fresh pork sausage	58.7	58.3	58.3	58.3	57.8				
a* values	Ground pork	17.9	27.0	14.5	15.8	15.0	0.5415	<0.0001	<0.0001	0.7554
	Fresh pork sausage	22.2	21.8	21.3	20.7	20.0				
b* values	Ground pork	19.7	19.6	19.2	19.0	18.7	0.4196	<0.0001	<0.0001	0.1225
	Fresh pork sausage	21.1	20.5	20.2	20.0	19.6				
Total microbial count (log_{10} (CFU)/g)	Ground pork	4.07	4.66	5.60	6.50	7.05	0.6266	0.1288	<0.0001	0.4286

Table 3.1 continued

	Fresh pork sausage	3.25	3.73	4.45	5.44	6.20				
B. thermospacta microbial count (log_{10} (CFU)/g)	Ground pork	2.70	3.27	4.21	4.72	5.62	0.6511	0.7763	<0.0001	0.1847
	Fresh pork sausage	2.29	3.26	3.88	4.84	5.40				
Lactic acid microbial count (log_{10} (CFU)/g)	Ground pork	3.97	4.60	5.61	6.10	6.58	0.6255	0.1334	<0.0001	0.3796
	Fresh pork sausage	3.24	3.38	4.44	5.16	5.82				

SEM= standard error of mean.

Table 3.2: The means for antioxidant analyses for treatment effect (ground pork and fresh pork sausages) during a five day display period at 4°C (three batches)

	Fresh sausages		
Antioxidant enzymes	Ground pork	Fresh pork sausage	SEM
Catalase (U/g meat)	194[a]	164[b]	4.480
GSHx (U/g meat)	0.825[a]	0.680[b]	0.0388
SOD (IU/g meat)	45.9[a]	37.8[b]	2.108
TEAC (mmole Trolox/L)	0.016[a]	0.014[b]	0.0003

Means with the same letter in the same row is not significantly different ($p<0.05$).
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Table 3.3: The means for antioxidant analyses for day effect of both ground pork and fresh pork sausage during a five day display period at 4°C (three batches)

	Catalase (U/g meat)		SOD (IU/g meat)		TEAC (mmole Trolox/L)	
Day	Mean	SEM	Mean	SEM	Mean	SEM
1	281[a]	14.34	47.8[a]	2.074	0.017[a]	0.0002
2	223[b]	7.567	44.4[ab]	1.997	0.016 [b]	0.0003
3	154[c]	4.406	42.8[bc]	1.745	0.015 [c]	0.0003
4	140[d]	5.181	40.2[c]	1.484	0.014 [d]	0.0003
5	96.1[e]	7.555	34.1[d]	1.247	0.013 [e]	0.0003

Means with the same letter in the same column are not significantly different ($p<0.05$).
The multi-treatment comparisons were made using the pdiff method. . SEM= standard error of mean.

Table 3.4: Correlation coefficients of chemical, colour and microbial analyses for ground pork during a five day display period at 4°C (three batches)

	Catalase	GSHPx	SOD	TEAC	MetMb	L*	a*	b*	TSA	STAA
GSHPx	-0.35									
SOD	0.62*	0.19								
TEAC	0.65*	-0.65*	0.03							
MetMb	0.38	0.23	0.55*	-0.16						
L*	-0.19	-0.73*	-0.12	0.50*	-0.60					
a*	0.66*	0.09	0.67**	0.26	0.68**	-0.44				
b*	0.56*	-0.12	0.18	0.36	0.58*	-0.50*	0.84***			
TSA	-0.87***	0.14	-0.76**	-0.34	0.55*	0.34	-0.86***	-0.73**		
STAA	-0.93***	0.26	-0.73*	-0.35	-0.57*	0.35	-0.82***	-0.72**	0.97***	
MRS	-0.92***	0.13	-0.71*	-0.31	-0.54*	0.42	-0.81***	-0.73**	0.97***	0.96***

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table 3.5: Correlation coefficients of chemical, colour and microbial analyses for fresh pork sausages during a five day display period at 4°C (three batches)

	Catalase	GSHPx	SOD	TEAC	MetMb	L*	a*	b*	TSA	STAA
GSHPx	0.18									
SOD	0.53*	0.41								
TEAC	0.74**	-0.12	0.19							
MetMb	0.35	-0.42	-0.28	0.59**						
L*	0.27	-0.49	-0.50	0.63**	0.57**					
a*	0.80**	-0.03	0.66**	0.06	-0.02	-0.59**				
b*	0.77**	-0.22	0.43	0.32	0.27	-0.28	0.92***			
TSA	-0.78**	-0.15	-0.69**	-0.39	-0.02	0.34	-0.86***	-0.80***		
STAA	-0.84***	0.03	-0.48	-0.45*	-0.12	0.32	-0.87***	-0.85***	0.91***	
MRS	-0.83***	-0.23	-0.75**	-0.30	0.07	0.42	-0.89***	-0.80***	0.98***	0.92***

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

3.1.2 Colour analyses of pork patties

Meat colour is often measured by using tricolourmetric measurements such as Lab system, where L*, a* and b* are the colour coordinates for lightness, redness and yellowness. This system has been found to be appropriate to characterise meat colour and the a*-value most notably seems to correlate to sensory properties of the meat (Jeremiah *et al.*, 1972). In general, discolouration correlates with an increase in metmyoglobin during the display period with a large drop in all other colour parameters (L*, a* and b*) (Viana *et al.*, 2005). The colour intensity (chroma) also falls as the meat becomes dull and hue angles increase (Young and West, 2001)

3.1.2.1 Relative amount of metmyoglobin

While the values for the relative amount of metmyoglobin was significantly different on days 1 and day 5 (Table 3.1), the treatment effect was not significant (Table 3.1 and 3.6) over the 5 day display period. In this study, a significant elevation in the amount of metmyoglobin was observed from day 2 to day 3 and from day 3 to day 4 (Table 3.7). The elevation of the amount of metmyoglobin during storage *post mortem* may be caused by both the reduction of metmyoglobin reductase activity (Zhu and Brewer, 1998) and oxygen consumption rate (Atkinson and Follet, 1973). Therefore, as time increased, the metmyoglobin accumulated so that a more pronounced grey-brown colour was observed on the surface of both the ground pork and fresh pork sausage patties. McKenna *et al.* (2005) stated that low metmyoglobin values (~1.40-1.30) were observed on day 1 for all muscles. In this study, the metmyoglobin values on day 1 for both ground pork and fresh pork sausage patties was not as high as the value that was reported by McKenna *et al.* (2005) because pork has lower amount of myoglobin than beef.

When meat starts to loss its colour, the meat become less red which mean that a* values decrease and the relative amount of metmyoglobin increases. Metmyoglobin formation has been shown to negatively correlate with a* due to oxidation of myoglobin during meat storage (Renerre *et al.*, 1996). In my research, this observation was seen in ground pork patties but not in fresh pork sausage patties. The relative amount of metmyoglobin in fresh pork sausage patties did not have significant correlation with a* values (Table 3.5) but did for the ground pork alone (Table 3.4).

Stewart *et al.* (1965) established a linear relationship between relative amount of metmyoglobin and K/S ratio from a mixture of beef and pork cuts. They reported that ratio values of 1.40, 1.30, 1.20, 1.10, 1.00 and 0.90 corresponded with approximately 0%, 12%, 23%, 34%, 46% and 59% metmyoglobin, respectively. Moreover, consumers begin discriminating against beef steaks when approximately 20% metmyoglobin is present (Renerre and Labas, 1987). According to Hood (1975), who used a linear relationship established by Stewart *et al.* (1965), consumer discrimination against beef steak occurs at a ratio between 1.20 and 1.24, depending on initial ratio values. In this study, however, the K/S ratio at day 1 was only 1.07 (Table 3.7) which has the highest a* values (Table 3.7). Therefore, the established value for % metmyoglobin for beef is somewhat different from the pork used in this study. The correspondence of the K/S values and % metmyoglobin in pork must be established, along with the colour sensory

analysis as determined by panelists, in order to find the discrimination point of % metmyoglobin.

However, extrapolation from the results of previous studies with beef can be used to find the consumer discrimination point in this study. Hood (1975) reported that consumers could detect the difference in beef colour by 0.16 units or a 20% increase in metmyoglobin. Again, this value was established for beef, but then if the initial value of metmyoglobin at day 1 for beef (1.30, McKenna *et al.*, 2005) is compared with pork sausages (1.07) as found in this study (table 3.7), the consumer could be expected to be able to discriminate a change in product colour in fresh pork sausages at a K/S ratio of 0.13 [(1.07/1.30) x 0.16]. Therefore, consumers should start to be able to discriminate the colour difference in ground pork pork patties in this study from day 1 to day 5 because the K/S value was 0.14 which is higher than 0.13 (Table 3.8) but not in fresh pork sausage patties since the difference in K/S ratio from day 1 to day 5 was only 0.09 < 0.14 (Table 3.1). This result suggests that the fresh pork sausage patties had a longer shelf-life in comparison to ground pork patties.

Salting could decrease the ability of the endogeneous antioxidant enzymes to control superoxide anion and peroxide concentration (Kanner *et al.* 1991), thus the salted sausages would tend to discolour more vigorously than the ground pork. However, this result was not observed in this study, suggesting that lemon powder or sodium erythorbate might have a colour enhancing effect that reduced the prooxidant activity of salt.

3.1.2.2 L* values

The L* values of the colour measurement of the pork patties were significantly different over time (Table 3.1); while the ground pork patties had higher L* value than the fresh pork sausage patties, this different was not significant (Table 3.6). Seyfert *et al.* (2007) indicated in their study that time had a predictable effect as colour stability during display where generally the L* values increased during storage, the a* values decreased during storage while b* values increased from day 1 to day 3. Then, it was followed by only a slight change from day 3 to day 6 with no pattern. This observation was also supported by Lindahl *et al.* (2006a). On the other hand, a decrease in lightness (L*) was observed throughout the storage period in different atmosphere as studied by Viana *et al.* (2005). In this study, as indicated in Table 3.7, overall the L*values tended to be rather stable in beef steaks from different muscles at 2±2 °C. This result also agrees with McKenna *et al.* (2005) who concluded that changes in L* values were very subtle over a typical retail display time course. Bradford *et al.* (1993) similarly stated that L* values and b* values were not affected by the storage time on fresh pork sausage at 5-7°C.

Table 3.6: The means for colour analyses for treatment effect of both ground pork and fresh pork sausages during a five display period at 4°C (three batches)

Colour measurement	Ground pork	Fresh pork sausage	SEM
Relative amount of metmyoglobin	1.05[a]	0.989[a]	0.022
L*values	58.6[a]	58.3[a]	0.3917
a*values	21.2[a]	16.5[b]	0.2493
b*values	20.3[a]	19.2[b]	0.0003

Means with the same letter in the same row is not significantly different ($p<0.05$).
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Table 3.7: The means for colour analyses for day effect of both ground pork and fresh pork sausages during a five display period at 4°C (three batches)

	Relative amount of Metmyoglobin		L*values		a*values		b*values	
Day	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	1.07[a]	0.0164	58.8[a]	0.3182	20.1[a]	0.2951	20.4[a]	0.1245
2	1.05[a]	0.0188	58.5[b]	0.2637	19.4[b]	0.1878	20.0[b]	0.1245
3	1.02[b]	0.0217	58.4[b]	0.3106	18.9[c]	0.2035	19.7[c]	0.1245
4	0.998[c]	0.0225	58.4[b]	0.2933	18.3[d]	0.1815	19.5[d]	0.1245
5	0.956[c]	0.0165	58.1[c]	0.2563	17.5[e]	0.1689	19.1[e]	0.1245

Means with the same letter in the same column are not significantly different ($p<0.05$).
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

3.1.2.3 a* values

Both treatment and day effect were significant during the whole display period (Table 3.1). Ground pork patties had lower a* values over the period of day 1 to day 5 than did fresh pork sausage patties, and that this difference was significant (Table 3.6). The redness of the meat decreased over the display period and this may due to the autoxidation of the myoglobin to metmyoglobin which was clearly reflected by the elevation of the relative content of metmyoglobin throughout the display period. The a* values significantly correlated with b* values for both the ground pork (0.84) and lemon (0.92) patties, as seen in Table 3.4 and 3.5, respectively. The positive b* values indicate yellowness in meat colour, and this yellowness had a strong relationship with the redness in this study (Table 3.3 and 3.4).

Consumers are supposedly able to perceive a difference in a* value of 0.6 – 0.9 depending on the light source (Zhu and Brewer, 1999). If the consumers are able to discriminate the discolouration when a difference in a* values is equal to 0.9, then the

consumers would detect the discolouration at day 2 for ground pork patties and at day 3 for fresh pork sausage patties in this study (Table 3.1). This result thus suggests that fresh pork sausage patties had a longer shelf life than ground pork patties.

3.1.2.4 b* values

As seen for a* values, the effect of treatment and time effect on b* values were significant (Table 3.1). The data clearly showed that the b* values for fresh sausage was higher than ground pork at each time (Table 3.6), whereas the b* values significantly decreased every day (Table 3.7). As mentioned previously, Seyfert *et al.* (2007) indicated b* values increased from day 1 to 3. Moreover, Bradford *et al.* (1993) also stated that L* values and b* values were not affected by the storage time on fresh pork sausage at 5-7°C. Their result is in contradiction with this study, where the b* values decreased during the display period from day 1 to day 5. While there is a still a lack of consensus with respect to b* value, it is important to note that the majority of studies have been conducted using beef.

3.1.3 Microbial analyses

Fresh sausages are perishable products because they provide a good environment for microorganisms to grow in terms of their pH and A_w. Fresh sausages usually have a pH value not lower than 5.5 and A_w equal to or greater than 0.97 (Cocolin *et al.*, 2004). Therefore, fresh sausages provide a relatively good environment for microbial growth. Fresh sausages undergo no fermentation, drying or thermal processing so during the storage at 4°C, hygienic quality of the raw materials are critical factors affecting the final value of the products (Cocolin *et al.*, 2004). The only hurdle to spoilage of this class of product is refrigeration, and a shelf life of 10 days can be expected when these products are stored at 4°C (Cocolin *et al.*, 2004). It is clearly very important to minimize the initial bacterial load in fresh sausages during preparation stage.

The initial mesophilic bacterial count in raw pork is usually ~10 CFU/g (Kalliopi *et al.*, 2005). In this study, the total microbial count was about 10^3 CFU/g after processing (and before storage) for both ground pork and fresh pork sausage patties (Table 3.8). This value is higher than reported by Kalliopi *et al.* (2005) because in this study the meat was processed. In fresh sausage, the spoilage point is reached at a microbial load of 7-8 $\log_{10}$CFU/g (Lambert *et al.*, 1991). At 10°C, spoilage occurs after 6 days, whereas at 0°C, spoilage is delayed to more than 3 weeks (Lambert *et al.*, 1991). Therefore, in this study, the shelf-life of the fresh sausages should be more than 6 days since they were displayed at 4°C. The total microbial count only reached a maximum of 6.6 $\log_{10}$ CFU/g in ground pork patties by day 5 (Table 3.8).

Table 3.1 shows that the number of organisms which grew on TSA, STAA and MRS plates were significantly ($p<0.05$) different over time. There was no significant difference between the ground pork and fresh pork sausage patty (Table 3.8);

even though.the ground pork patty had higher microbial count than fresh pork sausage patty. The growth rate for all 3 different microbial counts during storage period at -1°C from day -6 to day 0 was slower in comparison to the growth rate during display period at 4°C from day 1 to day 5 (Table 3.8). This result suggests that the microbial growth is faster at higher temperature.

In general, meat that is stored under aerobic conditions will be spoiled more rapidly by Enterobacteriacea, lactic acid bacteria, *Pseudomonas spp.*, and *Brochothrix thermosphacta* (Borch *et al.*, 1996). Specifically, *B. thermosphacta* and coliforms have been found to be the predominant bacteria associated with the spoilage of pork under all temperatures and atmospheres. *Pseudomonas spp.* only dominates under aerobic atmospheres (Liu *et al.*, 2006). In spoiled meat, *Lactobacillus sakei/curvatus* and *Leuconostoc mesenteroides* are example of lactic acid bacteria, whereas *Enterobacter amnigenus* and Hafnia are examples of Enterobacteriaceae (Borch *et al.*, 1996). When microorganisms grow in the same environment, they definitely interact with each other and their interactions during storage can play an important role in spoilage.

In the presence of Enterobacteriaceae and Pseudomonas, *B. thermosphacta* showed the same growth trends as when it was cultured alone (Russo *et al.*, 2006). However, in the presence of lactic acid bacteria, *B. thermosphacta* strains tended to yield lower counts after 5 days of incubation (Russo *et al.*, 2006). Table 3.9 shows that the total microbial count was higher than lactic acid bacterial counts, which in turn were higher than *B. thermosphacta* counts; this trend was evident in both ground pork and fresh pork sausage patties. This result suggests that lactic acid bacteria were the dominant microorganisms in both ground pork and fresh pork sausage patties, as they made up the majority of the total microbial counts. This is also in agreement with Holley *et al.* (2004), who showed that after 48 hours the *B. thermosphacta* counts were 2 log cycles lower in the presence of lactic acid bacteria than without the lactic acid bacteria. These statements are also supported by Russo *et al.* (2006) who found that the lactic acid bacteria counts were higher than *B. thermosphacta* counts during the whole incubation period. In contrast, Cocolin *et al.* (2004) showed the growth of *Leuconostoc mesenteroides* and *B. thermosphacta* remained high until at the end of the monitoring period and they concluded these organisms were among the most active populations during their study.

This antagonistic activity of lactic acid bacteria toward *B. thermosphacta* may be due to the production of acid by the lactic acid bacteria; these organisms are known to lower the pH of the environment, as well as compete for the same substrate for growth (Greer and Dilts, 1994). It is thought, in this case, that the production of organic acid by lactic acid bacteria might be balanced by the end products of the metabolism of enterobacteria and fecal enterococci, such as ammonia as well as free amino acids produced by proteolytic activity of yeast species.

Other reasons for the inhibition of *B. thermosphacta*, besides the drop in pH during display period, include an antagonistic effect from lactic acid bacteria, whose rapid growth result in consumption of the same nutrients required by *B. thermosphacta*.

Alternatively, Gardner (1981) suggested that the production of H_2O_2 by lactic acid bacteria may be the reason for *B. thermosphacta* inhibition. Lactic acid bacteria lack peroxidase (Kono and Fridovicj, 1983), so in the presence of lactic acid bacteria under aerobic conditions, hydrogen peroxidase could accumulate and inhibit the growth of *B. thermosphacta*. It is important to note, however, that even though the growth of the lactic acid bacteria is very rapid, they did not contribute significantly to meat spoilage because of reduced secretion of metabolites with less offensive characteristics than those produced by aerobic bacteria such as *B. thermosphacta* (Viana *et al.*, 2005).

In this study, the numbers of all types of microorganism were always higher in the ground pork patties than in the fresh pork sausage patties, even though this difference was not significant at the $p<0.05$ level (Table 3.9). However, a difference at $p<0.1$ still can be used to asses trends. This observed trend may be caused by the fact that the addition of ingredients inhibited microbial growth. For example, the addition of salt would tend to reduce the water activity available for microbial growth. Moreover, the addition of lemon juice powder decreased the overall pH of the fresh pork sausage patties, again acting to limit the growth of certain microorganism such as *B. thermosphacta*. Meat that has a pH 5.8 with restricted oxygen availability due to packaging material is a good environment for the growth of *B. thermosphacta* (Dainty and Hibbard, 1980). Campbell *et al.* (1979) reported that *B. thermosphacta* was unable to grow on beef under aerobic conditions at pH less than 5.8. Moreover, Blickstad and Molin (1983) showed that *B. thermosphacta* failed to grow aerobically at pH 5.3 at 8°C. In this study, as indicated in Figure 3.3, the pH values were lower in fresh pork sausage (~5.6) than in ground pork (~5.8); therefore, the ground pork patties provided a better environment for *B. thermosphacta* to grow and accordingly, the *B. thermosphacta* counts were higher in ground pork patties than in ground pork patties (Table 3.8).

In fresh pork sausage patties, all of the counts on TSA, STAA and MRS (-0.86, -0.87, -0.89, respectively) had a significant negative correlations only with a* values (Table 3.5). Moreover, in ground pork patties, the microbial counts significantly correlated with the relative amount of metmyoglobin and a* values (Table 3.4). In general, it can be concluded that microbial growth caused meat discolouration in terms of lowering a* values and increasing the relative amount of metmyoglobin during the display period.

Aerobic bacterial growth can accelerate oxidation of meat pigments that can lead to meat discolouration (Lawrie, 1991) by increasing the rate of myoglobin autoxidation in aerobic conditions (Renerree, 2000) through the elevation of oxygen consumption and the reduction of partial oxygen pressure to the level critical for myoglobin oxidation (Cheah and Ledward, 1997). A reduction in oxygen partial pressure (~10 mm Hg) on the meat surface as a result the microbial growth over time could cause discolouration on meat surface due to the accumulation of metmyoglobin that would be formed more rapidly under lower oxygen partial pressure (Labadie, 1999).

Microorganisms also have the ability to change the meat pH and produce amino acids and amines through proteolysis and glycolysis that further induce the myoglobin autoxidation (Hedrick *et al.*, 1989). Thus, any factors that can enhance microbial growth

will also increase of the rate myoglobin oxidation rate (Sofos *et al.*, 2000) which then leads to meat discolouration. However, it is important to note that anaerobic bacteria do not generally cause meat discolouration (Kropf *et al.*, 1986) and lactic acid bacteria have no influence on development of brown colour (Sofos *et al.*, 2000).

3.1.4 Redox potential analysis

Redox potential (E_h) is a measurement of the ease by which a substance gains or loses electrons. E_h is measured in millivolts. A fully-oxidized standard oxygen electrode will have an E_h of +810 mV at pH 7.0, 30° C (86° F), and under the same conditions, a completely reduced standard hydrogen electrode will have an E_h of –420 mV. The E_h is dependent on the pH of the substrate; normally, the E_h is taken at pH 7.0 (Reichart *et al.*, 2006).

Different groups of meat products have different redox potential values. The E_h of raw meat is in the negative range, roughly around -200 to -300 mV (Rödel and Scheüer, 1999b). Meat products, however, have a completely changed redox system than raw meat. Meat processing and the addition of ingredients and additives to meat products shift the redox to a more positive range; for example, fermented raw meat products have E_h values ranging between 100 to 200 mV, and heated meat products have E_h values ranging between -25 to 100 mV (Rödel and Scheüer, 1999b). As indicated in Figure 3.1 and Figure 3.2, the E_h values on the processed pork patties were on the positive side (~ 200±50 mV) initially, due to the grinding process that incorporates O_2 in the meat system. *Post mortem* meat redox potential decreases very rapidly from initial values of 100 mV down to -100 mV or – 200 mV (Ahn and Maurer, 1989). This reduction was seen in this study but the reduction did not exceed – 50 mV (Figure 3.2).

Many extrinsic factors influence microbial growth, including the oxidation-reduction potential or E_h (Tabatabai and Walker, 1970; Motilva *et al.*, 1992; Barnes and Ingrams, 1956). Therefore, the measurement of redox potential the meat products could be intended to assess microbial activity that also related to colour stability of the meat product. According to Kukec *et al.* (2002), redox potential measurement could assess the ability of life of microorganisms, growth as well as the physiological activity in defined environment. Indeed, it is very important to know the parameters that influence the survival and growth of microorganisms in the products to predict the shelf-life of meat products (Rödel and Lücke, 1989). Microbial growth that caused spoilage usually occurs on the surface of meat products, and E_h on the surface of the meat is more readily affected by the microbial activity than the E_h in the middle of the meat products.

There was a significant negative correlation of the redox potential to the total count of microorganisms and the distinctively lower potential values after storage of 14 days in samples with visible sign of spoilage (Hoffman, 1974). In this study, as seen in Figure 3.1 and 3.2, the E_h on the surface of the both patty treatments decreased more significantly nearer to the end of display period; this reduction of redox potential value likely reflects the more rapid growth of microbial at this time. Kukec *et al.* (2002) also indicated that exponential phase growth was followed by a fast drop of redox potential.

Table 3.8: The means for microbial analyses for day effect of both ground pork and fresh pork sausages during display period a five at 4°C (three batches)

	Total microbial count (log_{10} CFU/g)		*B. thermosphacta* count (log_{10} CFU/g)		Lactic acid bacteria count (log_{10} CFU/g)	
Day	Mean	SEM	Mean	SEM	Mean	SEM
5	6.1^{a}	0.243	5.508^{a}	0.336	6.2^{a}	0.277
4	6.0^{b}	0.227	4.776^{b}	0.328	5.6^{b}	0.277
3	5.0^{c}	0.350	4.046^{c}	0.207	5.0^{c}	0.277
2	4.2^{d}	0.313	3.266^{d}	0.265	4.2^{d}	0.277
1	3.7^{e}	0.256	2.496^{e}	0.363	3.6^{e}	0.277

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Table 3.9: The means for microbial analyses for both ground pork and fresh pork sausages during display a five period at 4°C (three batches)

	Fresh sausages		
Microbial analysis	Ground pork	Fresh pork sausages	SEM
Total microbial count -TSA (log_{10} CFU/g)	5.6^{a}	4.6^{a}	0.285
B. thermosphacta count - STAA (log_{10} CFU/g)	4.1^{a}	3.9^{a}	0.396
Lactic acid bacteria count -MRS (log_{10} CFU/g)	5.4^{a}	4.5^{a}	0.366

Means with the same letter in the same row are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Benedict *et al.* (1975) noted that the significant decrease of both pH and E_h after 8 and 10 days of storage may have been caused by an alteration of bacterial metabolism. In general, the energy source for microbial growth is via the biological oxidation of complex macromolecules in meat, a process that results in the formation of a more reducing environment. Metabolic oxygen consumption further contributes to this effect and also forms reducing compounds in the surrounding microbial microenvironment (Reichart *et al.*, 2007). Metabolites resulting from bacterial activity are generally water soluble and would affect the pH and E_h effectively.

The shape of the redox potential curve is characteristic of the type of microorganisms numerically dominant during bacterial growth (Reichart *et al.*, 2007). Tabatabai and Walker (1970) stated that initial E_h and dissolved oxygen content of the culture medium played an important role in microbial growth. In their study, they showed that pure cultures of *Clostridium perfringens* and *Pseudomonas fluorescens* grew better in media that had an initial E_h of 200 mV (with oxygen present) than in the media that had initial E_h of 40 mV (without oxygen present). Barnes and Ingram (1956) demonstrated that *Cl. perfringens* only initiated growth in fresh meat after the E_h value dropped to below -36 mV. In contrast, Rödel and Lücke (1989) did not observe any effect of initial E_h on the growth of *Bacillus subtilis* and *Basillus licheniformis*. Regardless, most studies show a decrease in redox potential during exponential growth of various bacteria under both anaerobic and aerobic conditions (Tabatabai and Walker, 1970). As far as this study is concerned, the later statement can be concluded, but any further interpretation regarding the optimum initial E_h could not be derived.

Gradients of E_h may exist from the surface to the center of the foods when inward gaseous diffusion is restricted (Brown and Emberger, 1980). Therefore, in the center of the patty, a more anaerobic environment exists than on the surface of the meat patty, usually reflected by a more negative E_h. In this study, the pork patties were only 10 mm in thickness, so the difference in the E_h tended to be not as noticeable. The penetration depth depends on the rate of oxygen diffusion, rate of oxygen consumption and the oxygen partial pressure (O'Keeffe and Hood 1982; Renerre 2000). *Post mortem*, meat continues to consume oxygen for the mitochondrial electron transport chain. Therefore, myoglobin must compete with mitochondrial respiratory system for oxygen that diffuses into the meat from the atmosphere. The oxygen consumption rate (OCR) decreases over time because of the depletion of substrate coenzymes (NADH) and the degradation of enzymes involved in mitochondrial respiration (O'Keeffe and Hood 1982). Therefore, besides microbial activity, redox potential can also serve as an indicator of mitochondrial respiration *post mortem* during the display period through the redox measurements from microelectrodes that were located in the center of the patties. When the OCR decreases in meat patties, the E_h should increase since more oxygen is now available, as seen in Figure 3.2. This elevation of redox potential at the end of the display period may indicate that more oxygen has become available and this oxygen now can bind myoglobin to form oxymyoglobin in fresh pork sausage patties.

Difficulties arise in obtaining accurate redox measurements because it is hard to obtain equilibrium in redox at the point of measurement. The irregularity of the E_h profile and

the variations between measurements in the fresh sausages may also be due to the heterogeneity of texture of the sausages (FDA, 2001); thus, it is hard to obtain reproducible E_h results among replicates. These values can be highly variable depending on changes in the pH of the food, microbial growth, packaging, partial pressure of oxygen in the storage environment, and ingredients and composition, such as, protein, ascorbic acid, reducing sugars and oxidation level of cations. Therefore, in order to a get consistent measure of absolute redox potential, the affecting variables must be kept constant. Another important factor is the poising capacity, or buffering capacity, of the food system; this factor influences the extent that a food resists external affected changes in E_h (FDA, 2001). The poising capacity of the food will be affected by oxidizing and reducing constituents in the food as well as by the presence of active respiratory enzyme systems. Lastly, irregularities in the E_h measurement may also be affected by volatile components that affect the redox potential in the process before reaching the equilibrium (H_2S, H_2, O_2 and SO_2). Therefore, it is somewhat difficult to get reliable absolute redox potential values, thus if E_h measurements are not performed in combination with other measure of microbial growth potential, incorrect conclusions can be derived. Even though the absolute redox potential value is hard to measure, in this study, the absolute redox values are not needed in order to observe the trend of the redox values during storage.

3.1.5 Proximate analysis and pH measurement of meat patties

In this study, proximate analyses were done for controlin the variation of the raw picnic shoulder from the suppler. In this study, the fat content of each sausage batch was kept constant at around in order to minimize variation in terms of the source of radical that could affect the efficacy of the endogenous antioxidant enzymes and colour stability. As shown in Table 3.10, the protein content had a 6.7% coefficient of variation. Therefore, the variability among batches was small which was not the source of much variation.

In figure 3.3, day -7 represents the production day (slaughter), and the pH at this point was lower than the other days. Kalliopi *et al.* (2005) reported that the pH in the fresh sausages had an initial value of 5.61± 0.007 and did not change significantly during the display period. As shown in Figure 3.3, the pH values of ground pork patties were higher than the fresh pork sausage patties. Over time during both storage and display, the pH of fresh pork sausage patties did not really change, whereas the pH values for ground pork patties were seen to increase rapidly, especially from day 4 to day 5 of the display period.

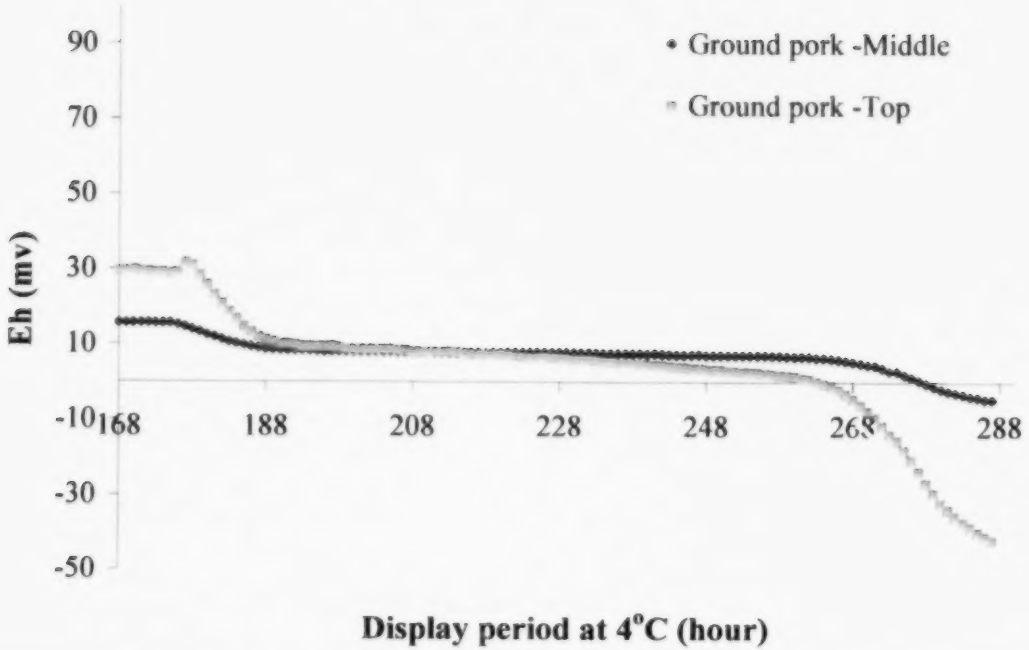


Figure 3.1: The effect of storage period at -1°C and display period at 4°C (illuminated with 850 lux – 1100 lux light) on the redox potential (Eh) of ground pork patties with microelectrodes positioned 2 mm from the patty surface (Top) and 5 mm from the patty surface (Middle), starting from day 1 (168 hours) to day 5 (288 hours) of the display period.

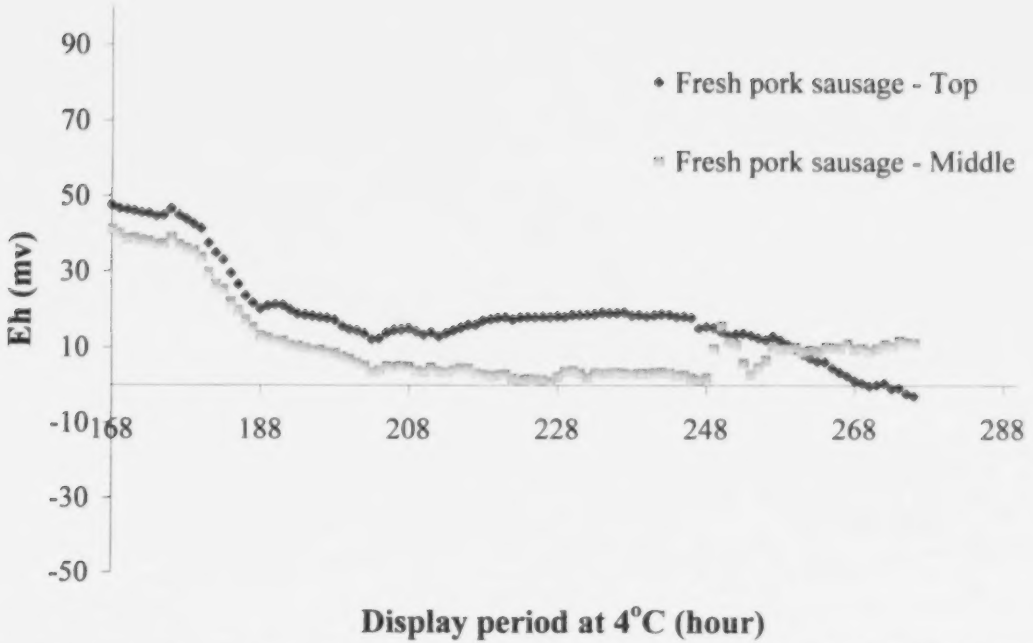


Figure 3.2: The effect of storage period at -1°C and display period at 4°C (illuminated with 850 lux – 1100 lux light) on the redox potential (Eh) for fresh pork sausage patties with the microelectrodes positioned 2 mm from the patty surface (Top) and 5 mm from the patty surface (Middle), starting from day 1 (168 hours) to day 5 (288 hours) of the display period.

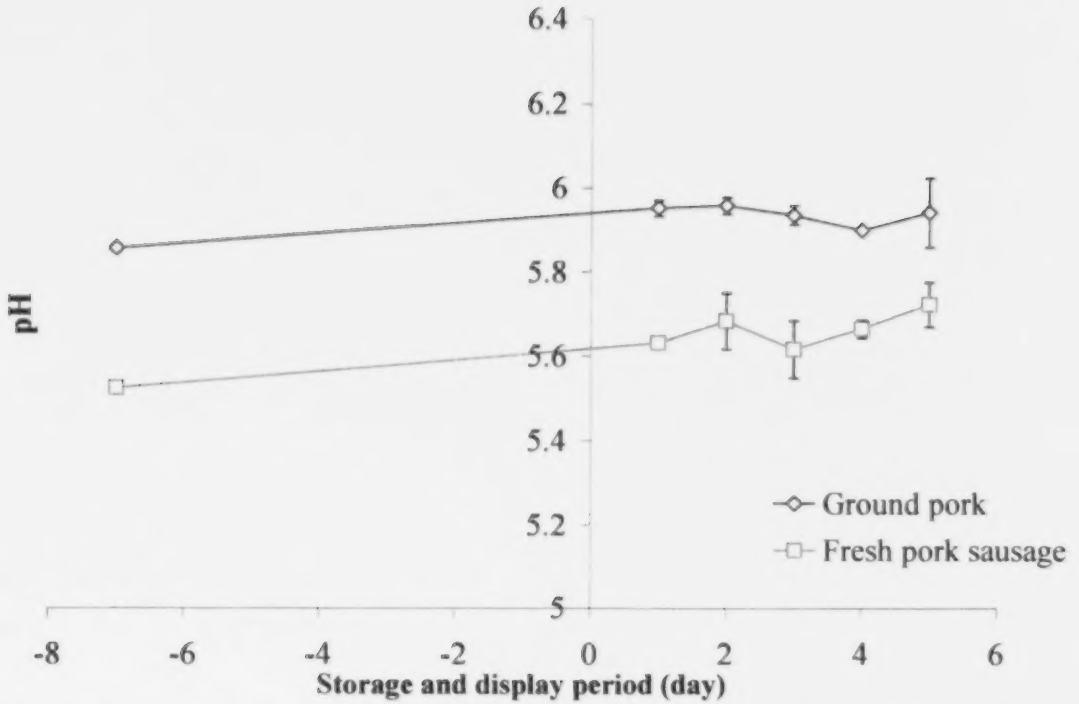


Figure 3.3: The pH values of both ground pork and fresh pork sausage during the storage and display period (4°C and illuminated with 850 lux – 1100 lux light) at constant 4°C (three batches).

Table 3.10: The means, standard deviation and coefficient of variation (CV) of proximate analysis of the picnic shoulder boneless pork obtained from Maple Leaf Foods, shown for three different batches

	Proximate analysis		
	Moisture (%)	Fat (%)	Protein (%)
Batch 1	65.5	14.6	16.9
Batch 2	64.1	16.6	16.2
Batch 3	65.5	15.1	18.4
Average	65.0	15.4	17.2
STD	0.8	1.0	1.1
CV (%)	1.2	6.7	6.5

STD = standard deviation, CV = coefficient variation

3.2. STUDY 2 - Assessing the effect of non-meat ingredients on the stability of fresh pork sausage over time at 4°C

3.2.1 Antioxidant activity

As indicated in Table 3.11, the day effect was found to be significant for catalase, GSHPx and SOD activities for all the types of fresh pork sausages (A, B, C and D), with decreases in the respective values over time (Table 3.12 and 3.13). As known, the oxidative stability of muscle foods can be altered by food processing operations and food additives. In general, enzyme activities are affected by electrolytes and ionic strength (Richardson and Hyslop, 1985). NaCl is known to have prooxidant effects at concentrations of 0.5% - 2.0% (Rhee *et al.*, 1983) and functions by compromising the ability of endogenous antioxidant enzymes to inhibit lipid oxidation of meat during storage (Lee *et al.*, 1997). Maximal inactivation of catalase, glutathione peroxidase and superoxide dismutase in stored salted ground pork were 2% NaCl, 0.5 and 1.0% NaCl and 1.0% NaCl, respectively (Lee *et al.*, 1997). However, the addition of other ingredients, such as sodium erythorbate and lemon juice powder, as part of the fresh sausage formulation could increase the oxidative stability of the meat products. Coronado *et al.* (2002) concluded that the addition of sodium erythorbate in sausage's formulation increased the oxidative stability of the sausages due to its antioxidant capacity. As indicated earlier, erythorbate is an isomer of ascorbate and thus erythorbate functions as a reductant in food and biological systems. Ascorbic acid promotes iron reduction and solubilization, thus preventing the formation of insoluble ferric hydroxides. Moreover, it also can replace $O_2^{\cdot}$ which is dismutated to hydrogen peroxide and participates in the Fenton reaction (Khan and Martell, 1967).

In this study, there was no synergistic interaction between lemon juice powder and sodium erythorbate for all catalase, GSHPx and SOD activities (Table 3.11). The only significant interaction was between sodium erythorbate and catalase activity. This interaction means that the addition of 0.05% sodium erythorbate in fresh sausage B and D had a significant positive effect on catalase activity in comparison to fresh sausage A and C (Table 3.7), as indicated Table 3.14. In Figure 3.4, the decreasing rates of catalase activity for fresh sausage A and C can be seen to be lower than that for B and D. Antioxidants need to be sacrificed to protect against oxidation. Therefore, the different decreasing rate of catalase activity in Figure 3.4 could mean that sodium erythorbate could influence how the catalase acts as an antioxidant.

3.2.2 Colour

The product of myoglobin oxidation is metmyoglobin so in this study, the relative amount of myoglobin and a* values can be used as an indicator of myoglobin status. Fresh sausage formulation A decreased the most rapidly in terms of a* values and increased the most rapidly in terms of the relative amount of metmyoglobin oxidation (Table 3.15, Figure 3.5 and 3.6). This result indicates that NaCl may have increased the oxidation rate. It has previously been postulated that the prooxidant activity is associated with the ability of NaCl to alter the distribution and reactivity of iron (Decker and Xu,

Table 3.11: p values for: Lemon, Sodium erythorbate, Lemon x Sodium erythorbate, Day, and Lemon x Sodium erythorbate x Day

	***p* values**				
Variables	Lemon juice powder	Sodium erythorbate	Lemon juice powder x Sodium erythorbate	Day	Lemon juice powder x Sodium erythorbate x day
Catalase activity (U/g meat)	0.4341	0.0119	0.4653	<0.0001	0.2447
GSHx activity (U/g meat)	0.6992	0.1876	0.5120	<0.0001	0.8920
SOD activity (IU/g meat)	0.3867	0.9139	0.5958	0.0055	0.9674
Relative amount of metmyoglobin	0.1057	0.0986	0.4656	0.0002	0.7688
L* values	0.1489	0.0377	0.4008	0.0018	0.8919
a* values	0.256	0.0366	0.7020	0.0004	0.9469
b* values	0.3641	0.4546	0.5625	0.0015	0.9664
Chroma	0.1919	0.0514	0.9931	<0.0001	0.9958
Hue	0.5118	0.0661	0.5096	0.0015	0.8911
Total microbial count (log_{10} (CFU)/g)	0.4309	0.8582	0.9003	<0.0001	0.4749
B. thermosphacta microbial count (log_{10} (CFU)/g)	0.4117	0.9007	0.7362	<0.0001	0.7522
Lactic acid microbial count (log_{10} (CFU)/g)	0.4544	0.4730	0.5112	<0.0001	0.1525

Table 3.12: The mean all types of fresh sausages A, B, C and D for catalase, SOD and GSHPx activity during a ten day display period at 4°C for (three batches)

	Catalase activity (U/g meat)		SOD (IU/ g meat)		GSHPx (U/g meat)	
Day	Mean	SEM	Mean	SEM	Mean	SEM
-7	205^{a}	4.346	34.3^{a}	1.519	1.31^{a}	0.029
1	201^{a}	4.111	31.5^{ab}	1.519	1.28^{ab}	0.029
3	170^{b}	10.99	29.5^{bc}	1.519	1.21^{bc}	0.029
5	139^{c}	7.707	29.5^{bc}	1.519	1.16^{cd}	0.029
7	121^{d}	4.874	27.9^{bc}	1.519	1.15^{cd}	0.029
9	109^{d}	9.020	25.7^{c}	1.519	1.12^{d}	0.029

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

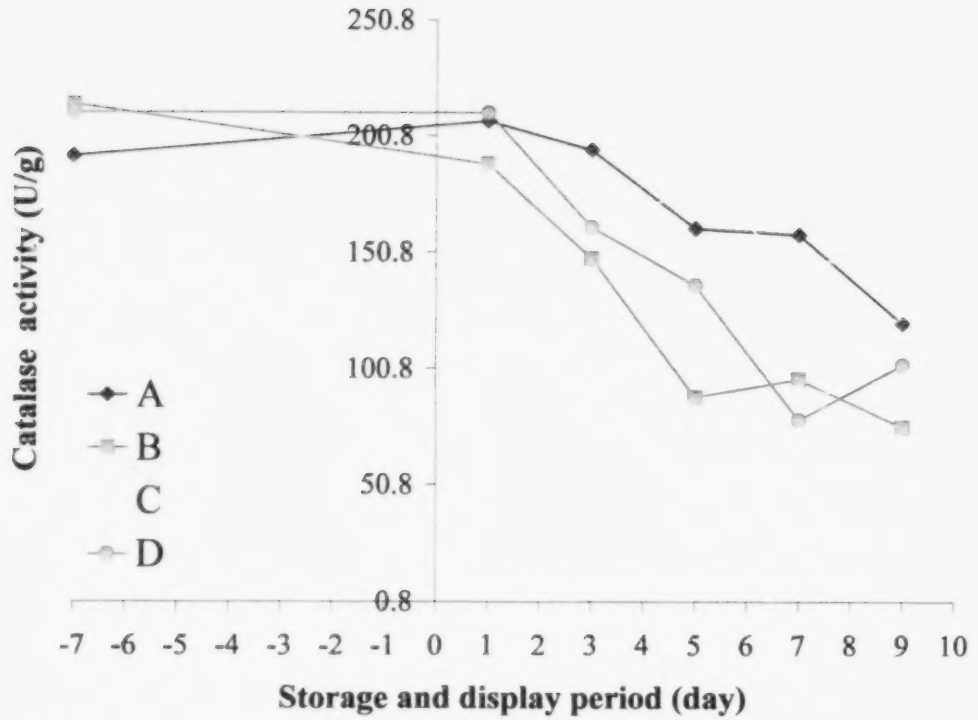


Figure 3.4: The effect of sodium erythorbate and lemon juice powder treatment (illuminated with 850 lux – 1100 lux light) on the L* values for fresh pork sausages A, B, C and D during storage (-1°C) and display period (4°C). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

Table 3.13: The effect of lemon juice powder and sodium erythorbate treatment on antioxidant activity in fresh pork sausages at production day and during a ten day display period at 4°C (three batches)

	Treatment		Display time						
Variables	Lemon juice powder	Sodium erythorbate	day p	day 1	day 3	day 5	day 7	day 9	SEM
Catalase activity	0.00%	0.00%	192	207	194	161	158	120	14.63
(U/g meat)	0.00%	0.05%	214	188	148	88.5	95.8	75.4	
	0.25%	0.00%	203	198	175	171	151	137	
	0.25%	0.05%	211	210	161	136[1]	102	78.4	
GSHx activity	0.00%	0.00%	1.27	1.24	1.21	1.21	1.20	1.13	0.0570
(U/g meat)	0.00%	0.05%	1.38	1.31	1.16	1.14	1.12	1.05	
	0.25%	0.00%	1.29	1.26	1.25	1.24	1.23	1.13	
	0.25%	0.05%	1.31	1.30	1.24	1.10	1.07	1.11	
SOD activity	0.00%	0.00%	27.8	32.4	33.7	28.6	23.8	27.3	3.038
(IU/g meat)	0.00%	0.05%	29.0	32.5	34.1	29.0	25.1	27.0	
	0.25%	0.00%	30.7	31.7	33.6	29.5	27.1	31.5	
	0.25%	0.05%	30.3	29.7	35.9	24.4	27.0	31.9	

The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Table 3.14: The means for antioxidant analyses for treatment effect of fresh pork sausages during a ten day display period at 4°C

Treatment		Catalase activity (U/g meat)		GSHPx (U/g meat)		SOD (U/g meat)	
Lemon juice powder	Sodium erythorbate	Mean	SEM	Mean	SEM	Mean	SEM
0.00%	0.00%	172[a]	9.345	1.21[a]	0.0232	28.9[a]	1.240
0.00%	0.05%	135.[b]	9.345	1.19[a]	0.0232	29.5[a]	1.240
0.25%	0.00%	172.[a]	9.345	1.23[a]	0.0232	30.7[a]	1.240
0.25%	0.05%	150[ab]	9.345	1.19[a]	0.0232	30.0[a]	1.240

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

1998), thereby affecting colour stability. However, the addition of sodium erythorbate and lemon juice powder in different type of fresh sausage patties could balance the negative effect of sodium chloride. Figure 3.5 indicates that fresh sausage A wich contains no sodium erythorbate and lemon juice powder has lowest a* during the whole storage and display period.

Sodium erythorbate, the less expensive salt isomer of ascorbic acid, performed similarly to ascorbic acid with respect to colour regardless of their distinct difference in pH (Mancini *et al*., 2006). Shivas *et al*. (1984) indicated that ascorbic acid is a reducing agent that could stabilize oxymyoglobin in meat products and delay lipid oxidation. It is capable of capable of maintaining myoglobin in a reduced ferrous state (Shivas *et al*., 1984). As indicated in Table 3.13, only sodium erythorbate had a significant positive effect on fresh pork sausage colour, particularly the L* values and a* values. The lightness of fresh sausages formulations B and D was significantly lower than A and C (Table 3.16 and Figure 3.7). The addition of 0.05% sodium erythorbate also increased the redness of fresh sausage formulations B and D (Table 3.16 and Figure 3.5). The decreasing trend for a* values was almost the same for all the treatments during the display period and its reduction slowed down on day 8 for all the treatments (Figure 3.5).

Table 3.15: The mean for all the types of fresh pork sausages (A, B, C and D) for relative amount of metmyoglobin, L* values, a* values, b* values, chroma and hue at production day and during a ten day display period at 4°C (three batches). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

	Relative amount of metmyoglobin		L* values		a* values		b* values		Chroma		Hue	
Day	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-7	1.25^{a}	0.0028	54.8^{d}	0.3266	23.6^{a}	0.4390	20.2^{ab}	0.6106	31.1^{a}	0.7245	40.4^{e}	0.3753
2	1.08^{b}	0.0199	57.7^{bc}	0.3101	20.4^{b}	0.5272	19.9^{a}	0.2184	28.6^{b}	0.4813	44.5^{d}	0.6529
4	1.04^{c}	0.0231	57.9^{b}	0.1583	19.2^{c}	0.6328	19.7^{a}	0.2321	27.5^{c}	0.5564	45.9^{c}	0.8356
6	0.927^{d}	0.0379	57.6^{c}	0.1467	17.1^{d}	1.037	19.2^{b}	0.3387	25.8^{d}	0.8667	48.9^{b}	1.561
8	0.801^{e}	0.0266	57.7^{bc}	0.2809	13.6^{e}	0.6227	18.2^{c}	0.2432	22.8^{e}	0.4043	53.5^{a}	1.369
10	0.757^{f}	0.0201	58.5^{a}	0.2687	12.9^{e}	0.3470	12.9^{c}	0.3434	22.1^{e}	0.2278	54.4^{a}	1.126

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Table 3.16: The effect of sodium erythorbate on L*values and a* values for 3 sausage batches during a seven days of storage period at -1°C and a ten day display period at 4°C for fresh sausage formulations with 0.00% sodium erythorbate (formulations A and C) and with 0.05% sodium erythorbate (formulations B and D)

Sodium erythorbate	L* values		a* values	
	Mean	SEM	Mean	SEM
0.00%	57.7[a]	0.1833	16.6[b]	0.6678
0.05%	57.1[b]		19.0[a]	

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

The reducing activity of ascorbic acid might improve muscle colour stability via metmyoglobin reduction (Lee *et al.*, 1999). Even though the antioxidant activity of ascorbic acid in meat and meat products on lipid oxidation have been found to be dependent on concentration, the presence of transition metal ions, and the presence of other antioxidants (Djenane *et al.*, 2002), the addition of 0.05% sodium erythorbate had a positive effect on meat colour stability during storage and display.

In this study, the lemon juice powder that was used contained 26.3 % citric acid. Citric acid is commonly added to food substances to chelate metal ions and control pH. Moreover, it has also been shown to have effects on food color (Bouchard and Merritt, 1979). Citric acid additionally reduces the pH of samples, which contributes to lightness (Sommers *et al.*, 2003), a statement supported by the result in this study as indicated in Figure 3.7 where fresh sausage formulation C had the highest L* values. According to Mancini *et al.* (2007), the effects of 1% and 3% citric acid was not as beneficial during display and 10% citric acid actually accelerated the discolouration process. In this study, only 0.25% lemon juice powder, which contains 0.07% citric acid, was used for fresh sausage formulations C and D, and this amount was not beneficial in delaying discolouration. At high pH, the haem iron is predominately in the Fe^{2+} state, and that low pH accelerates Fe^{2+} conversion to Fe^{3+} (Zhu and Brewer, 2002) so that pigment oxidation is promoted due to the denaturation of haem protein (Sammel and Clause, 2003). Even though the addition of citric acid causes no significant difference in pH (Table 3.19), it had a significant effect on colour in terms of a*values. The a* values of fresh sausage formulation B was higher than fresh sausage formulation C (Table 3.16). This result is in accordance with Nocolalde *et al.* (2006) who stated that at day 8, ascorbic acid-treated samples remained visibly redder than citric acid-treated samples (Nocolalde *et al.*, 2006). In addition, hue angle represents the degree of change from redness to yellowness (Chan *et al.*, 1996) and hue angle correlates well with subjective assessment of discolouration (Chan *et al.*, 1996). Figure 3.8 indicates that hue of fresh sausage formulation A was higher than C and C was higher than both B and D. This means that fresh sausage formulation A and C discoloured faster than B and D.

It has been reported that antioxidant activity of ascorbic acid is limited by the abundance of iron in meat because metal-induced oxidation of reducing agents often makes them inactive (Mancini *et al.*, 2007). Thus, the addition of chelators such as citric acid would improve ascorbic acid's efficacy in muscle food products. This suggests that the effects of ascorbic acid on colour may be improved by the addition of citric acid. Lund *et al.* (2006) found that the addition of citrate in combination with ascorbate lead to the reduction of lipid oxidation in minced beef patties in the dark up to 6 days at 4°C. In their study, after 3 days of storage only ascorbate and citrate preserved the red colour compared to no addition of antioxidant and addition of rosemary extract. However, according to Mancini *et al.* (2007) this relationship is not that straightforward since no synergistic effect was observed in their study whereas the use of only ascorbic acid was more beneficial than the combination of citric acid and ascorbic acid; an effect seen in this study (Table 3.16). Even though fresh sausage formulation D had the same low pH as fresh sausage C (Table 3.17 and Figure 3.9), the colour profile of fresh sausage formulation D was almost the same as fresh sausage B (Table 3.18). Therefore, it is more likely that the addition of sodium erythorbate balanced out the negative effect of lemon juice powder addition on meat colour due to its low pH than the other way around. In short, since there was no advantage in term of colour when both sodium erythorbate and lemon juice powder were added (at the concentrations used in this study), it would be more economical if meat manufacturers just added sodium erythorbate alone in their formulation at the concentration as indicated in this study.

Table 3.17: The effect lemon juice powder on pH for 3 sausage batches during a seven days of storage period at -1°C and a ten days display period at 4°C with 0.00% lemon juice powder (formulations A and B) and with 0.25% lemon juice powder (formulation C and D)

	pH	
Lemon juice powder	Mean	SEM
0.00%	6.08[a]	0.0928
0.25%	5.83[a]	

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of m

Table 3.18: The effect of treatment (lemon juice powder and sodium erythorbate) on colour profile in fresh pork sausages at production day and during a ten day display period at 4°C (three batches)

	Treatment		Display time						
Variables	Lemon juice powder	Sodium erythorbate	day p	day 2	day 4	day 6	day 8	day 10	SEM
Relative amount of	0.00%	0.00%	1.25	1.00	0.94	0.80	0.68	0.67	0.0482
Metmyoglobin	0.00%	0.05%	1.25	1.10	1.08	0.95	0.81	0.77	
	0.25%	0.00%	1.25	1.09	1.04	0.93	0.84	0.80	
	0.25%	0.05%	1.25	1.14	1.11	1.02	0.88	0.80	
L* values	0.00%	0.00%	54.40	57.90	57.93	57.65	57.78	58.67	0.5167
	0.00%	0.05%	54.65	57.43	57.59	57.38	57.38	57.61	
	0.25%	0.00%	55.17	58.14	58.44	58.45	58.39	59.60	
	0.25%	0.05%	54.65	57.63	57.76	57.08	57.47	58.06	
a* values	0.00%	0.00%	23.68	18.18	16.74	14.25	11.17	11.11	1.280
	0.00%	0.05%	23.26	21.15	20.07	18.13	14.68	14.28	
	0.25%	0.00%	23.80	20.24	18.78	16.44	13.06	11.99	
	0.25%	0.05%	23.88	22.16	21.25	19.42	15.53	14.03	
b* values	0.00%	0.00%	20.16	19.23	18.89	18.53	18.25	18.33	0.7147
	0.00%	0.05%	19.75	20.01	19.72	19.01	17.86	17.56	
	0.25%	0.00%	20.51	19.84	19.50	19.17	18.02	17.58	
	0.25%	0.05%	20.51	20.70	20.56	20.03	18.53	18.26	
Chroma	0.00%	0.00%	31.10	26.49	25.28	23.43	21.40	21.50	1.164
	0.00%	0.05%	30.52	2.12	28.14	26.29	23.15	22.67	
	0.25%	0.00%	31.31	28.36	27.12	25.43	22.36	21.33	
	0.25%	0.05%	31.48	30.33	29.56	27.91	24.19	23.03	
Hue	0.00%	0.00%	40.34	46.79	48.67	52.77	58.73	58.55	2.186
	0.00%	0.05%	40.28	43.40	44.51	46.60	50.21	50.89	
	0.25%	0.00%	40.44	44.55	46.33	50.15	54.26	55.70	
	0.25%	0.05%	40.59	43.05	44.05	46.04	50.21	52.46	

Means with the same letter in the same column are not significantly different ($p<0.05$)
SEM= standard error of mean

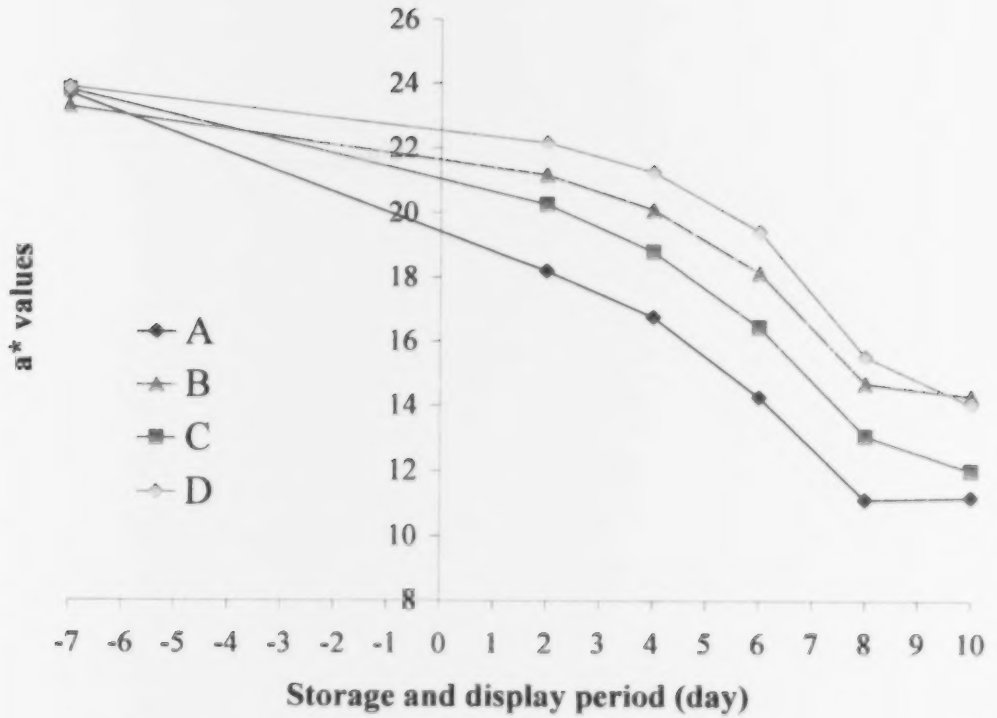


Figure 3.5: The effect of sodium erythorbate and lemon juice powder (illuminated with 850 lux – 1100 lux light) on the a* values for fresh pork sausage formulations A, B, C and D during storage (-1°C) and a ten day display period (4°C). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

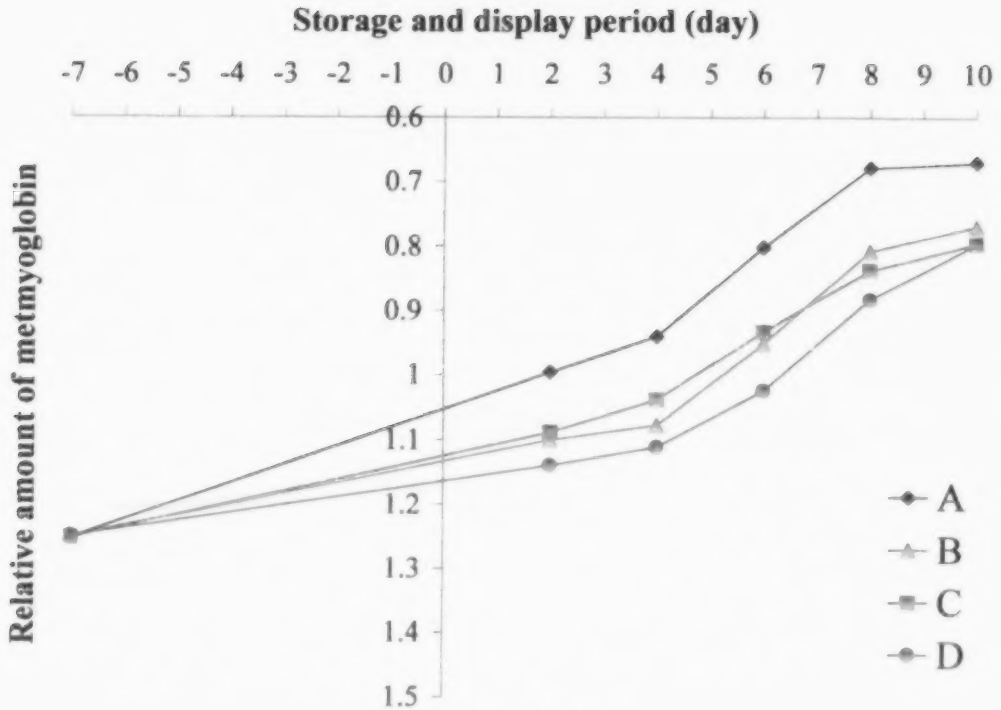


Figure 3.6: The effect of sodium erythorbate and lemon juice powder (illuminated with 850 lux – 1100 lux light) on the hue for fresh pork sausage formulations A, B, C and D during storage (-1°C) and a ten day display period (4°C). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

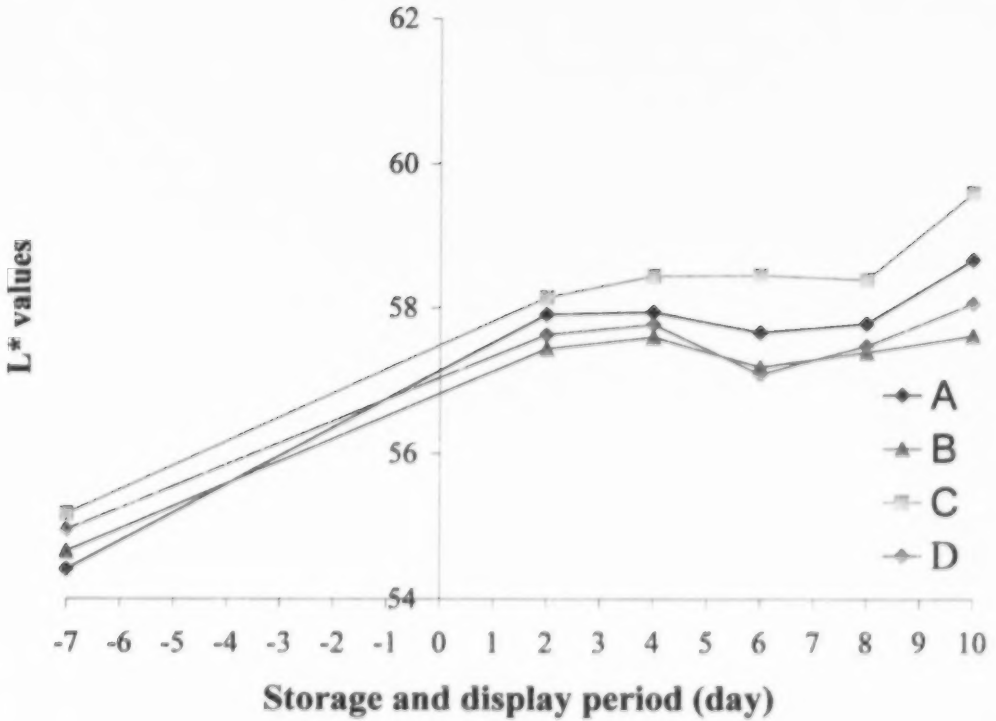


Figure 3.7: The effect of sodium erythorbate and lemon juice powder (illuminated with 850 lux – 1100 lux light) on L* values for fresh pork sausage formulations A, B, C and D during storage (-1°C) and 10 day display period (4°C). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

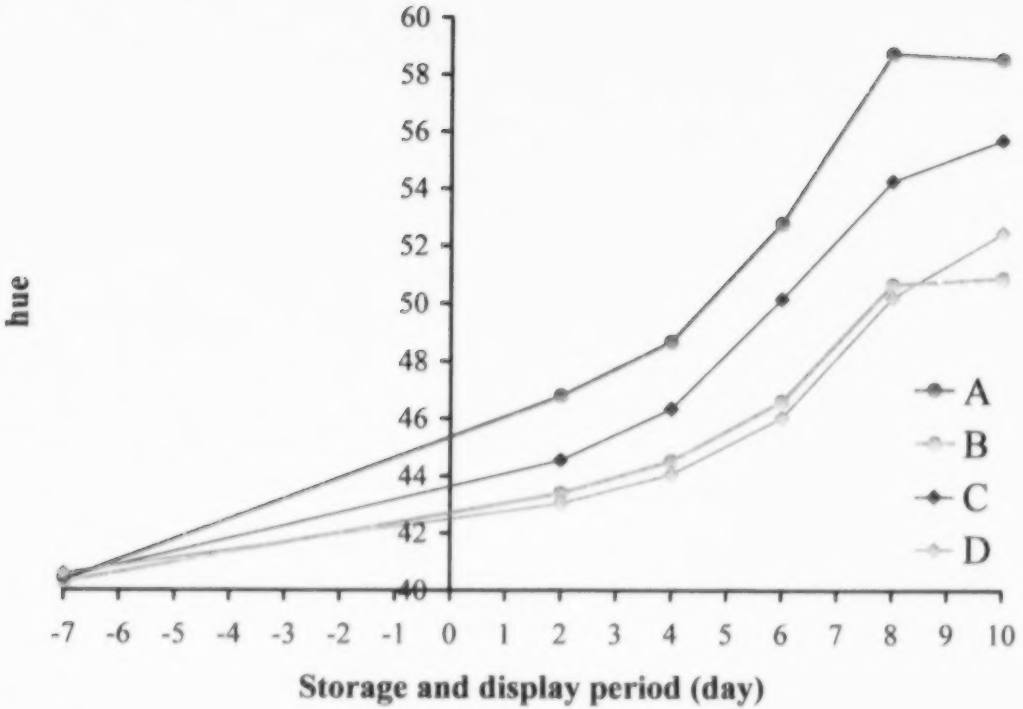


Figure 3.8: The effect of sodium erythorbate and lemon juice powder (illuminated with 850 lux – 1100 lux light) on the hue for fresh pork sausage formulations A, B, C and D during storage (-1°C) and a ten display period (4°C). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

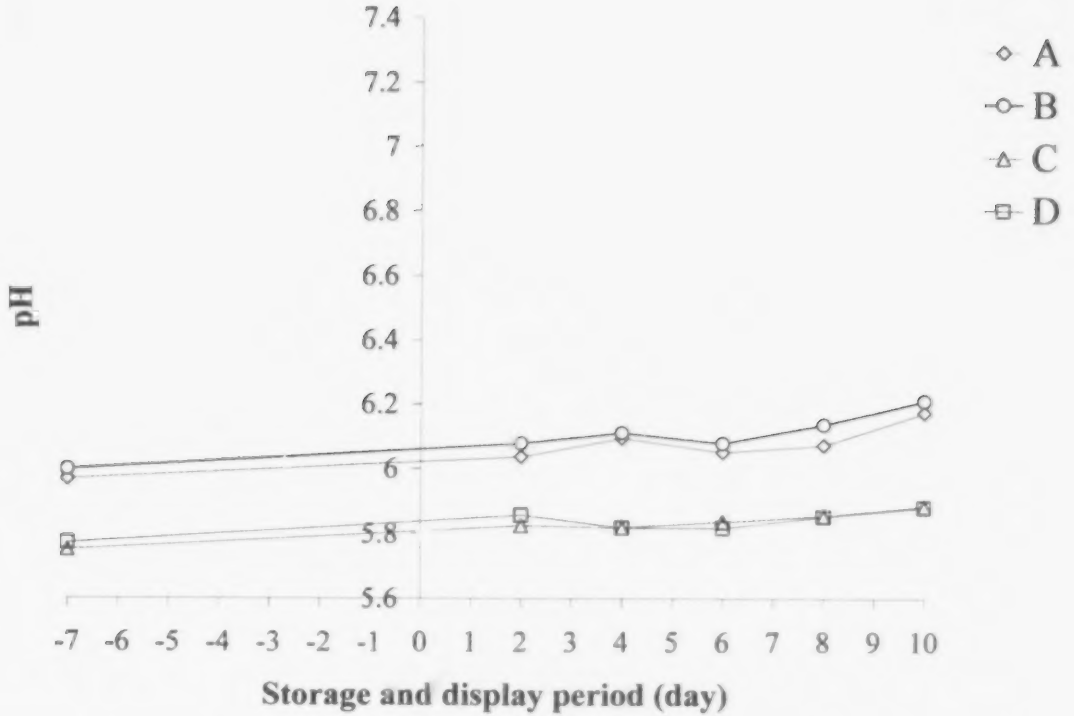


Figure 3.9: The pH values of pork patties during storage (seven days at -1°C in the dark) and display period (ten days at 4°C and illuminated with 850 lux – 1100 lux light). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

3.2.3 Microbiology

Gardner *et al.* (1967) reported that despite the high permeability of the packing materials used for this type of storage, some restriction of gaseous diffusion to and from packed meat occurs, resulting in a slight accumulation of CO_2. Carbon dioxide is inhibitory to *Pseudomonas* strains and its presence might account for the slightly higher incidence of *Brochothrix thermosphacta* on meat stored in gas-permeable packaging than on meat stored in air. In this study, however, lactic acid bacteria made up the majority of the total microbial counts.

Table 3.13 shows that there was no significant interaction between the non-meat ingredients and microbial count as also indicated in Table 3.19. However, there was a significant day effect on all classes of microbial counts with an increasing trend during the storage and display period (Tables 3.13, 3.20 and 3.21). Organic acids can inhibit microbial growth and are routinely used as preservatives (Sommers *et al.*, 2003). Buchanan and Golden (1994) indicated that citric acid exerted its antimicrobial effects via pH dependent- and independent-mechanisms. On the other hand, ascorbic acid did not inhibit microbial growth at various concentrations (Shivas *et al.*, 1984; Sahoo and Anjeneyulu, 1997). In this study, neither sodium erythorbate nor citric acid exhibited antimicrobial activity in comparison to the control. Additionally, there was no synergistic effect between sodium erythorbate and citric acid (Table 3.13 and 3.21). This result agrees with Rhee *et al.* (1997) who concluded that citrate and ascorbate did not reduce aerobic plate counts in cooked/aerobically refrigerated beef carrageenan patties.

Table 3.19: Means for microbial analyses for treatment effect of fresh pork sausages during ten days of display at 4°C (three batches)

Treatment		Total microbial count (log_{10} (CFU)/g)		*B. thermosphacta* microbial count (log_{10} (CFU)/g)		Lactic acid microbial count (log_{10} (CFU)/g)	
Lemon	Sodium erythorbate	Mean	SEM	Mean	SEM	Mean	SEM
0.00%	0.00%	6.7[a]	0.385	4.6[a]	0.135	5.6[a]	0.132
0.00%	0.05%	6.6[a]	0.385	4.5[a]	0.135	5.4[a]	0.132
0.25%	0.00%	6.4[a]	0.385	4.2[a]	0.135	5.4[a]	0.132
0.25%	0.05%	6.3[a]	0.385	4.3[a]	0.135	5.4[a]	0.132

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Table 3.20: Effect of lemon juice powder and sodium erythorbate treatment on microbial profile in fresh pork sausages during a ten display period at 4°C (three batches)

	Treatment		Display time						
Variables	Lemon juice powder	Sodium erythorbate	day p	day 1	day 3	day 5	day 7	day 9	SEM
Total microbial count	0.00%	0.00%	4.0	5.0	6.5	7.3	8.6	9.0	0.444
(log_{10}(CFU)/g)	0.00%	0.05%	4.0	4.6	6.2	7.3	8.4	9.0	
	0.25%	0.00%	4.0	4.7	5.8	7.2	7.7	8.6	
	0.25%	0.05%	3.7	4.5	5.8	7.2	8.3	8.5	
B. thermosphacta microbial count (log_{10} (CFU)/g)	0.00%	0.00%	1.9	2.9	4.4	5.5	6.4	6.7	0.526
	0.00%	0.05%	1.9	2.6	4.1	5.6	6.2	6.9	
	0.25%	0.00%	2.0	2.6	3.5	5.2	5.7	6	
	0.25%	0.05%	2.0	2.4	4.0	5.6	5.9	6.2	
Lactic acid microbial count (log_{10} (CFU)/g)	0.00%	0.00%	3.5	4.2	5.2	6.0	6.8	7.7	0.201
	0.00%	0.05%	6.5	3.9	4.9	5.9	6.8	7.3	
	0.25%	0.00%	3.5	4.1	4.8	6.1	6.5	7.2	
	0.25%	0.05%	3.4	3.8	5.1	5.9	6.5	7.4	

SEM= standard error of mean

Table 3.21: The mean of three batches (replicate) of each four formulations for total microorganisms, *B. thermosphacta* and lactic acid bacteria at production day and during a ten day display period at 4°C (three batches)

	Total microbial count (log_{10} (CFU)/g)		*B. thermosphacta* microbial count (log_{10} (CFU)/g)		Lactic acid microbial count (log_{10} (CFU)/g)	
Day	Mean	SEM	Mean	SEM	Mean	SEM
-7	4.0^{f}	0.194	1.9^{e}	0.088	3.5^{f}	0.045
2	4.7^{e}	0.137	2.6^{d}	0.089	4.0^{e}	0.046
4	6.1^{d}	0.311	4.0^{c}	0.163	5.0^{d}	0.081
6	7.3^{c}	0.299	5.5^{b}	0.316	6.0^{c}	0.075
8	8.3^{b}	0.149	6.0^{a}	0.317	6.6^{b}	0.106
10	8.8^{a}	0.175	6.4^{a}	0.415	7.4^{a}	0.161

Means with the same letter in the same column are not significantly different ($p<0.05$)
The multi-treatment comparisons were made using the pdiff method. SEM= standard error of mean

Microorganisms can metabolize glucose, lactic acid, certain amino acids, nucleotides, urea and sarcoplasmic proteins during storage. Microorganisms typically utilize the simpler constituents first, such as glucose, and thereafter use the more complex compounds, such as protein. In a study by Dainty (1996), a decrease in surface glucose concentration was first detected when cell numbers exceed $10^7/cm^2$, but 5 mm below the surface there was no reduction in glucose concentration. By the time bacterial numbers had reached $10^8/cm^2$, glucose was no longer detectable at the surface and decreased concentrations were detectable up to 10 mm lower. Bacteria then keep growing by utilizing various amino acids, as well as lactic acid. These secondary substrates do not initially become depleted at the surface. Shortly after this period, pH and ammonia concentrations began to increase at the surface and continue to do so until maximum cell numbers ($10^9/cm^2$) are reached (Dainty, 1996). Byproduct of protein consumption produces substances that cause spoilage in meat and meat products. Therefore, the addition of non-meat ingredients will not affect the microbiological profile unless more carbohydrate is added into the formulation to alter the metabolism of the microorganisms. Salt and sodium erythorbate do not contain any glucose but lemon juice powder contains 91.2 g of carbohydrate per 100 g of lemon juice powder. In this study, only 0.75 g of lemon juice powder was used in 1.5 kg batch meat batter, thus there is only 0.055 g of extra carbohydrate added in every 120 g fresh pork sausage patty. This amount was seemingly not sufficient to cause a significant difference in the growth of microbes in fresh pork sausage formulation C in comparison to fresh sausage formulations A, B and D.

3.2.4 Redox potential

Rödel and Scheuer (2000a) indicated that there is a correlation between redox potential and ingredients which are used in processing. According to their research, sodium ascorbate did not affect the pH of the sausage, but did lead to decreasing redox potential values. However, as the concentration of the sodium ascorbate reached 0.04 %, any further increase in concentration had little influence on the redox potential. In the present study, the initial redox potential in the middle of fresh sausage formulation A was 20 - 40 mV, and when 0.05% sodium ascorbate was added (fresh sausage B), the redox potential of the sausage was approximately – 40 mV – 50 mV (Figure 3.9). Since sodium ascorbate is a reductant, this result is not unexpected. Moreover, Holownia *et al.* (2003) indicated that raw chicken containing sodium erythorbate had a more negative redox potential than did the control, which means it had greater reducing conditions than control. Formulation D also contained 0.05% sodium erythorbate, and thus was also expected to have a low redox potential. Figure 3.10 shows that the redox potential of fresh sausage D was not as low as the redox potential of fresh sausage formulation B. This result may suggest that the interaction of lemon juice powder, which contained 26.3% citric acid and sodium erythorbate, increased the redox potential. The high reducing conditions allowed greater reactivity and interconversion of pigments that are not desirable from the perspective of meat colour because the *in situ* levels of reductants could control rates of metmyoglobin reduction to oxymyoglobin (Antonini and Brunori, 1971) and give a better colour stability in fresh pork sausage. The result from colour analyses showed that fresh pork sausages with sodium erythorbate had better colour and this may be due to a lower redox environment.

Citric acid does not have any reductant capacity but does function as a transition metal scavenger or synergist. Therefore, it does not have the ability to reduce the redox potential of the system but it does lower the pH of system (Figure 3.9). Figure 3.10 indicates that even though fresh pork sausage patty formulations A and C started at different redox potentials, eventually they came down to almost the same values. Moreover, fresh sausage formulations A and C had higher redox potentials in the patty center than patties B and D. This result suggested that citric acid did not affect the redox potential of the fresh pork sausage.

To assess the effect of microbial activity on redox potential and *vice versa*, redox potential measurements were made near the surface of fresh pork sausage patties. The formulation of different fresh sausages could affect the microbiological profile during storage and display because these ingredients could affect the redox potential and pH of the fresh pork sausages. According to Lemay *et al.* (2002), more positive redox values in a meat model system should be more favourable for growth aerobic organisms. Therefore, it was expected that fresh pork sausage A would have higher microbial growth than B and D, since A had higher middle redox potential. However, Figure 3.11 showed a different trend than what was expected, and fresh sausage C had the highest surface redox potential. While fresh sausage C also had a high redox potential, the pH was relatively low, a factor that could result in lower microbial growth than fresh sausage A. However, as shown in the previous section, neither the combination of 0.05% sodium erythorbate nor 0.25% lemon juice powder alone had a significant effect on microbial activity. Thus, the surface redox potential of fresh sausage formulations A, B, C and D should have similar trend but Figure 3.11, does not show that trend. Therefore, in this case, the surface redox potential does not correlate with the expected microbial activity.

3.2.5 Fresh pork sausage using DFD picnic boneless pork

During the third replication (batch) of this study, DFD (dark, firm, and dry) picnic boneless pork was used to make fresh pork sausages due to a change in meat supplier from Maple Leaf to Country Choice Meats (Duck Lake, SK). This batch was not included in other 3 batches so the analysis of DFD pork sausage was only based on one batch so the findings regarding DFD fresh pork sausage were only preliminary.The DFD pork sausages in this batch had a different microbial, colour profile and catalase activity than did the other batches. DFD meat has a higher ultimate pH (>6.0) and lower glucose content (Newton and Gill, 1981). Most bacteria favour a pH of around 7, so microbial growth was expected to be greater on the DFD fresh pork sausage. Figure 3.12 shows that DFD fresh pork sausages had higher initial pH values, making DFD a better environment for microbial growth; the initial microbial counts were higher for all time points and treatments using this material (Figure 3.13). Not only that, the microbial growth was more rapid in DFD fresh pork sausage than in normal fresh pork sausage (Figure 3.13). The substantial increase of pH (Figure 3.12) at day 4 may be due to the rapid increase in microbial growth, as indicated in Figure 3.13. The growth then slowed down at day 6 in DFD fresh pork sausages, suggestive of entry into the stationary phase.

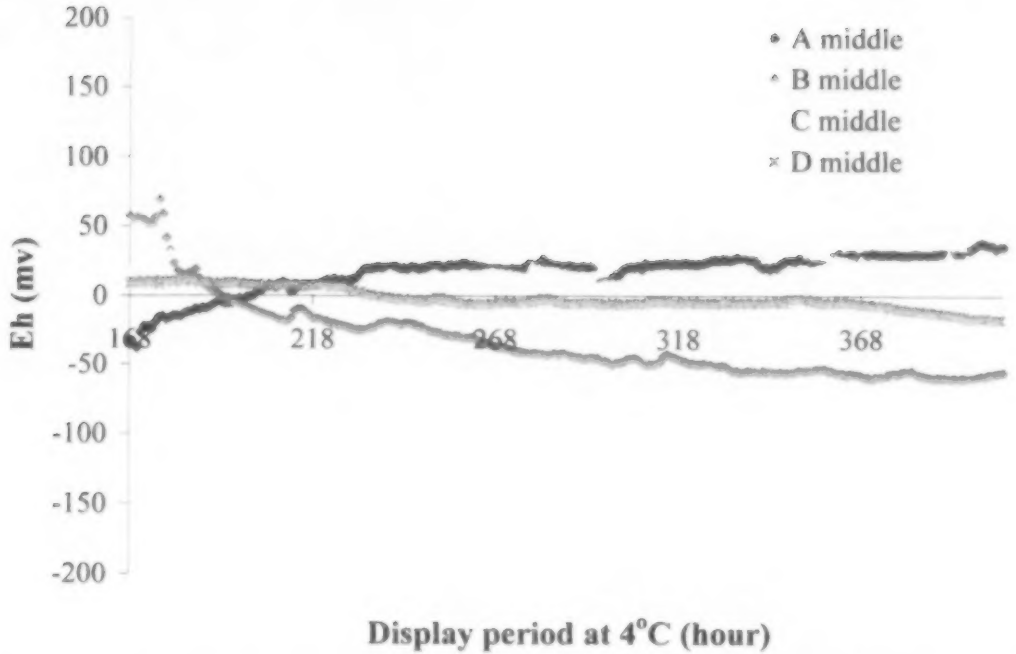


Figure 3.10: The effect of display period at 4°C (illuminated with 850 lux – 1100 lux light) on the redox potential (Eh) of fresh pork A, fresh pork B, fresh pork C and fresh pork D measured 5 mm from the surface of the patties (Middle) from day 1(168 hours) to day 10 (407 hours). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

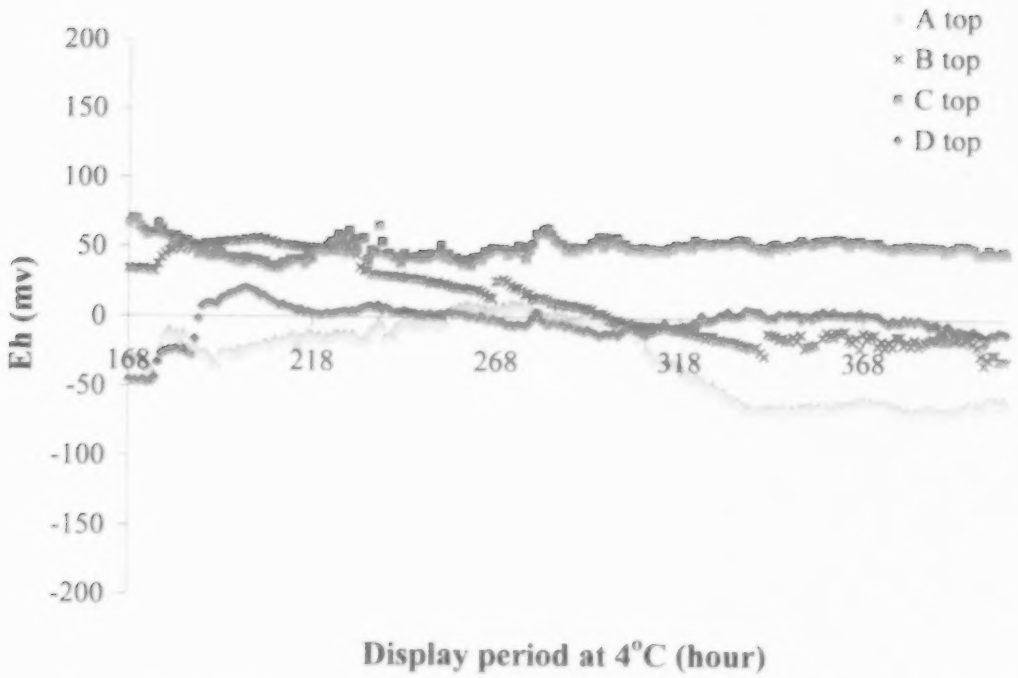


Figure 3.11: The effect of display period at 4°C (illuminated with 850 lux – 1100 lux light) on the redox potential (Eh) of fresh pork A, fresh pork B, fresh pork C and fresh pork D measured 2 mm from the surface of the patties (Top) from day 1(168 hours) to day 10 (407 hours). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

DFD meat has been known to spoil more rapidly than that of meat with normal pH, however the reasons are not completely clear. Newton and Gill (1981) suggested that DFD meat spoils more rapidly because it contains low concentration or even complete absence of glucose and glucose-6-phosphate in DFD meat means. This suggests that bacteria use amino acids and lactic acids are the initial substrates for growth. Consequently, DFD meat usually spoils more rapidly than normal meat. Newton and Gill (1978) compared the growth of *Pseudomonas sp.* on DFD beef with pH that was reduced with citric acid, as well as on DFD meat with glucose was supplemented to levels found in normal pH meat. Their results showed that off-odours were detected sooner and at lower microbial numbers in the samples lacking glucose regardless of pH. Therefore, the reason why DFD spoils faster is not due to microbial growth *per se*. Early spoilage is more likely due to the fact that microorganisms in DFD meat metabolize protein in earlier stage than in normal meat.

The DFD fresh pork sausages had higher L* values to begin with than normal fresh pork sausages, as indicated in Figure 3.14 b. Moreover, L* values increased up until day 4 at which time the L* values started to decrease. This trend did not happen for the normal fresh pork sausages (Figure 3.14 a). The DFD and normal fresh pork sausages had similar b* values to begin with but there was a sharp decrease of b* values at day 4 on DFD meat; the b* values then decreased at a slower rate for the rest of the display period.

In DFD meat, the reversion of discolouration occurred on day 4 of the display period (Figure 3.15 b). There are many reports that state that red colour and microbial growth are inversely related. Microbial growth can affect meat colour through bacterial oxygen consumption (Faustman and Cassens, 1990), so it can reduce the oxygen partial pressure to the point where metmyoglobin formation is maximized. However, further consumption of oxygen could then establish an environment permitting metmyoglobin reduction (Arihara *et al.*, 1993). Butler *et al.* (1953) observed that the elevation of metmyoglobin agreed with microbial logarithmic growth but further increases in microbial growth led to the reduction of metmyoglobin. In this study, colour reversion occurred at day 4 at which point the total microbial count was 8-9 $\log_{10}$ (CFU/g) (Figure 3.13); microbial growth was followed by an obvious increase in pH at day 4 (Figure 3.12). On the other hand, the cell counts on normal fresh pork sausages reached 8-9 $\log_{10}$ (CFU/g) at the end of display period (day 10); therefore, colour reversion was not observed in normal fresh pork sausages. Arihara *et al.* (1993) further stated that only aerobic bacteria have the ability to significantly reduce the oxygen tension at the meat surface and that facultative bacteria such lactobacilli or *B. thermosphacta* might not cause this colour reversion (Kropf *et al.*, 1986). Moreover, it has been reported that *Lactobacillus fermentum* JMC1173 can produce nitric oxide in media with no nitric oxide added (Lücke, 1985); and this intermediate converted metmyoglobin to a more desirable derivative, nitric oxide myoglobin (Morita *et al.*, 1998). However, this phenomena was not observed in this study so further study should be performed in the future.

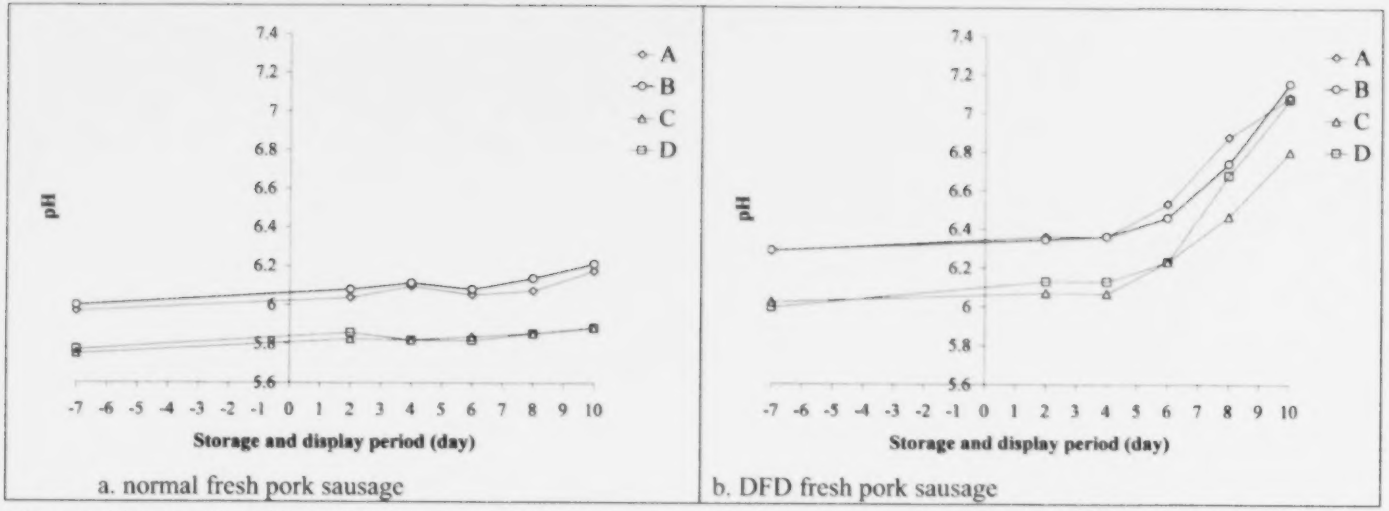


Figure 3.12: The pH profile during storage at -1°C and display at 4°C (illuminated with 850 lux – 1100 lux light) for a: normal fresh pork sausages and b: DFD fresh pork sausages. A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

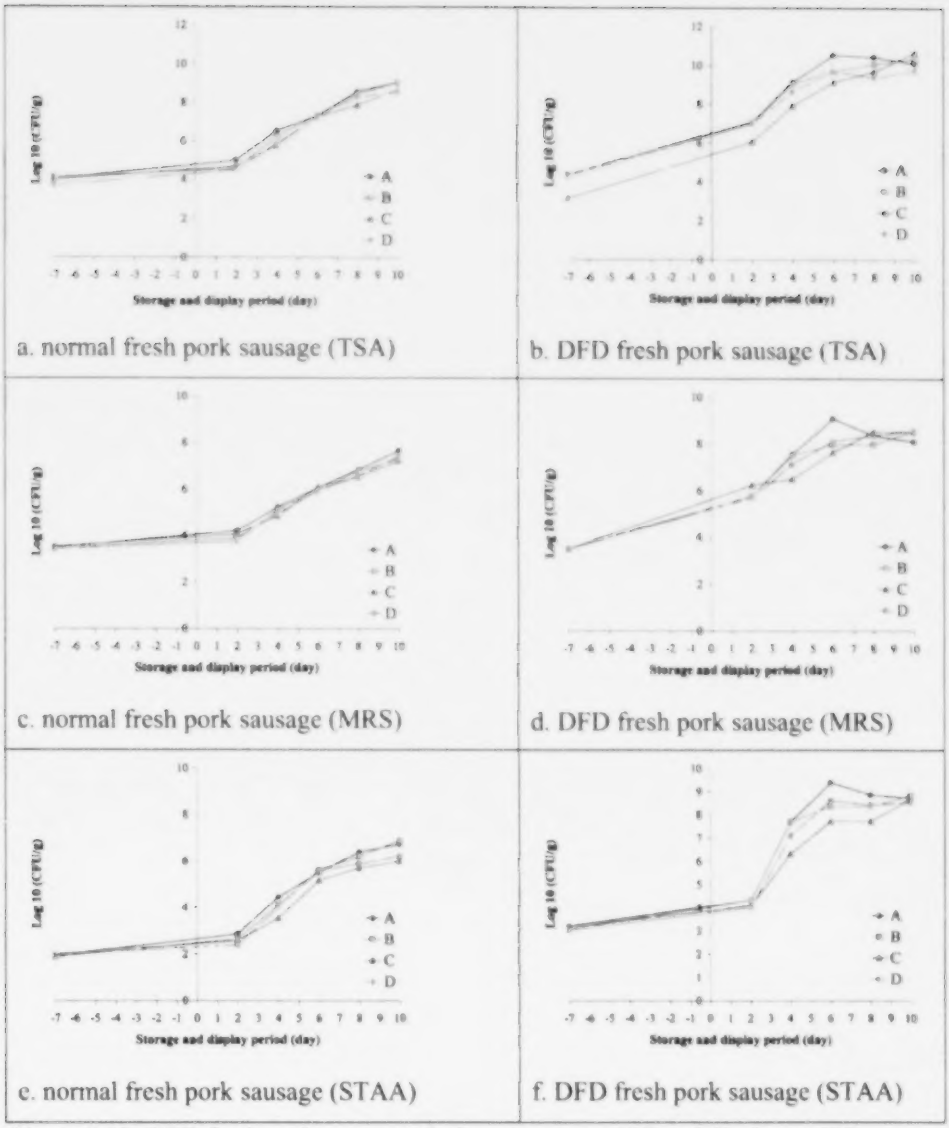
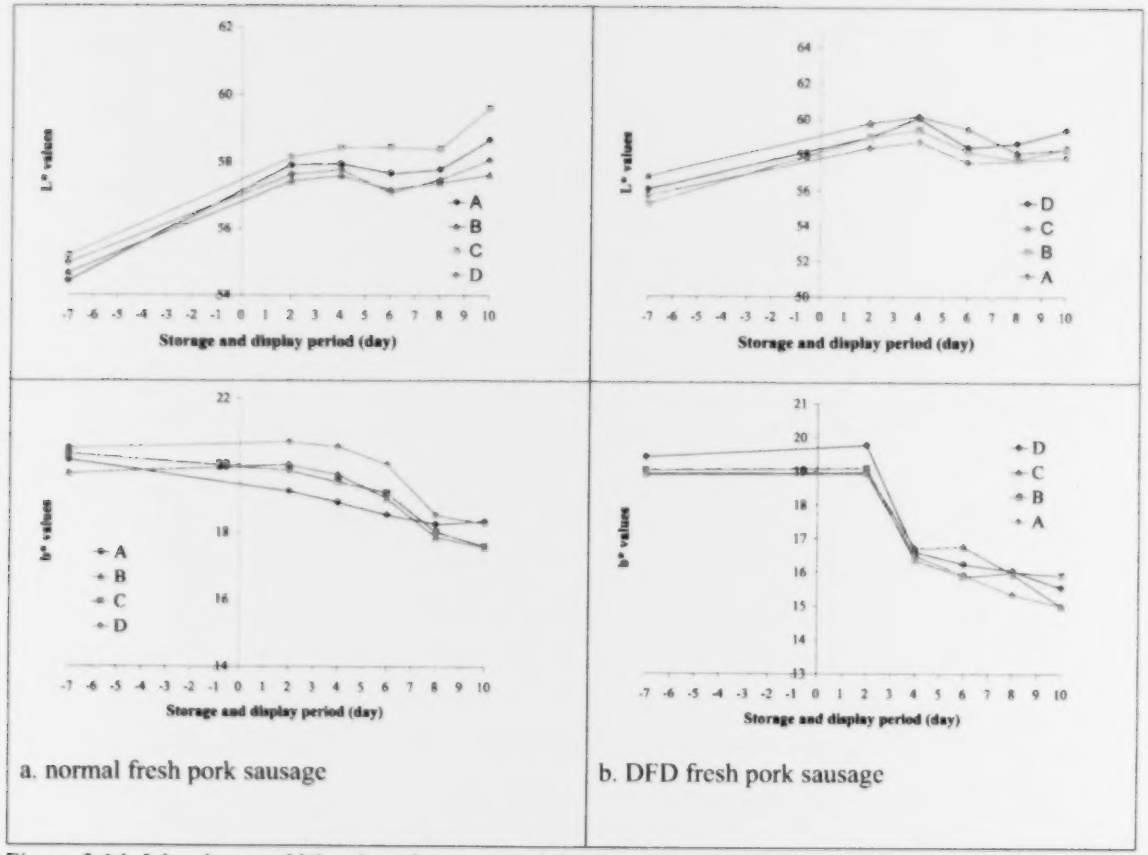


Figure 3.13: DFD fresh pork sausages during storage at -1°C and display at 4°C (illuminated with 850 lux – 1100 lux light) for a: total microbial count, b: lactic acid microbial count, c: B. thermosphacta microbial count. A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder



a. normal fresh pork sausage

b. DFD fresh pork sausage

Figure 3.14: L* values and b* values for a: normal fresh pork sausages and b: DFD fresh pork sausages during storage at -1°C and display at 4°C (illuminated with 850 lux – 1100 lux light). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

For antioxidant analyses, the most obvious difference in sausage formulations was for the parameter, catalase activity. Figure 3.14 shows that there was a plateau in catalase activity that started on day 3 to day 7, followed by a significant drop the following day. There is no publication that reports the relation of colour reversion and antioxidant activity. Further investigation is necessary in order to clarify the full mechanisms on how antioxidant activity affects colour reversion.

3.2.6 The effect of retail light on antioxidant activity, colour and microbial activity

Display period and storage period are often used interchangeably but they don't actually have the same definition. Display period is the period when the products are offered under lighting in the refrigerated retail display. Storage period is when stored products are held in the dark under refrigeration and usually not for sale. In this study, the regular fresh pork sausages were stored for 7 days at a constant -1°C in the dark and then they were displayed at a constant 4°C for 10 days under constant 850 – 1100 lux illumination. In order to determine the effect of the illumination on the antioxidant capacity, colour and microbial activity of fresh pork sausages, formulation D sausages (0.05% sodium erythorbate and 0.25% citric acid) under normal display condition were compared to formulation DD fresh pork sausages under dark display conditions at 4°C for 10 days.

Ramsbottom *et al.* (1951) found that fluorescent lighting at 600 to 2000 lux intensity had no effect on colour during 3-day display. In their study, steaks stored in the dark for 3, 5 or 7 days prior to display had a display life similar to those placed in display without any prior dark storage time. Likewise, in this study, the illumination of fluorescent light with intensity of 850 – 1100 lux did not affect the colour profile, antioxidant activity and microbial activity of the fresh pork sausage (Table 3.22). Eventhough the different in colour profiles of both fresh sausages D and DD is not significant, the a* values can still be used for analysing the trend (Table 2.22). Fresh pork sausages that were displayed in the dark (DD) has better red colour during the display period than the ones that were displayed under the light (D). Therefore, if the intensity of the light that is used for display is increased, there is a possibility that it can affect more damage in meat colour. However, it has been reported by Ramsbottom *et al.* (1951) found that fluorescent lighting at 600 to 2000 lux intensity has no effect on colour during 3-day display.

It has been reported that display lighting could affect the rate of discolouration due to the temperature elevation at the meat surface (Gould, 1963) and also due to photochemical changes (Bertelsen and Skibsted, 1987).The temperature of the meat surface has been shown to increase proportionally with increased light intensity under both incandescent and deluxe cool white fluorescent lights (Calkins *et al.*, 1986). Higher temperatures at the meat surface speed up deteriorative influences on meat color such as oxidation and microbial metabolism. Moreover, photochemical effects are caused by certain wavelength energies that excite one or more molecules and initiate or catalyze such reactions as oxidation which leads to a change in the meat pigment, myoglobin, causing discoloration.

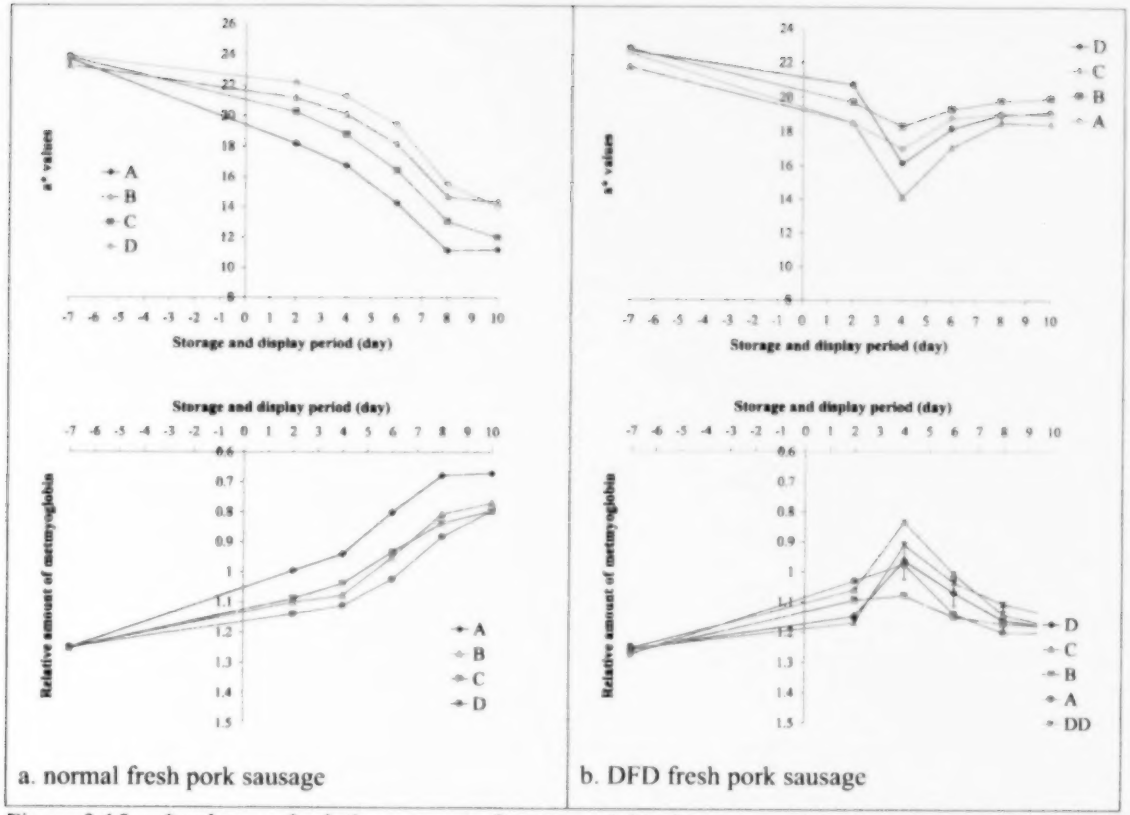
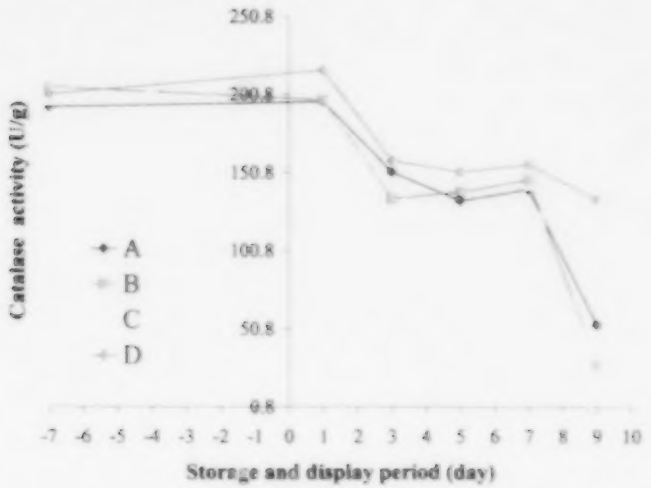


Figure 3.15: a * values and relative amount of metmyoglobin for a: normal fresh pork sausages and b: DFD fresh pork sausages during storage at -1°C and display at 4°C (illuminated with 850 lux – 1100 lux light). A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

a. Normal fresh pork sausages



b. DFD fresh pork sausages

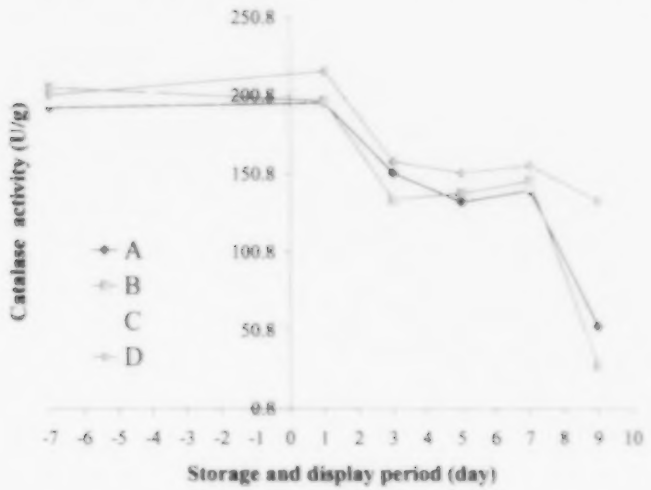


Figure 3.16: Catalase activity for a:normal fresh pork sausages and b: DFD fresh pork sausages during storage at -1°C and display at 4°C (illuminated with 850 lux – 1100 lux light. A = 0% sodium erythorbate and 0% lemon juice powder, B = 0.05% sodium erythorbate, C = 0.25% lemon juice powder, D = 0.05% sodium erythorbate and 0.25% lemon juice powder

The temperature of the meat surface increases proportionally with increased light intensity under both incandescent and deluxe cool white fluorescent lights. Higher temperatures at the meat surface speed up deteriorative influences on meat color such as oxidation and microbial metabolism. It has been reported that microbial growth appeared to be enhanced by illumination with fluorescent light (Djenane *et al.*, 2001). Moreover, photochemical effects are caused by certain wavelength energies that excite one or more molecules and initiate or catalyze such reactions as oxidation which leads to a change in the meat pigment, myoglobin, causing discoloration. In this study, none of the light-related factors significantly affected the colour profile, antioxidant activity and microbial activity. Furthermore, product illumination also did not affect the pH of the fresh pork sausage during the storage and display period (Figure 3.17). Lastly, further study must be done in order to analyze the effect of light intetnsity during display period and endogeneous antioxidant activity since there is a no publication has been published regarding this.

Table 3.22: The effect of light on antioxidant activity, colour and microbial activity in fresh pork sausages during a ten day display period at 4°C (three batches)

Variables	Treatment	Display time						SEM	*p* values treatment
		day p	day 1	day3	day 5	day 7	day 9		
Catalase activity	Light	208			150		110	15.30	0.1491
(U/g meat)	Dark	208			189		159		
GSHx activity	Light	1.29			1.12		1.14	0.0710	0.7187
(U/g meat)	Dark	1.29			1.19		1.12		
SOD activity	Light	31.2			33.1		30.6	3.038	0.4955
(IU/g meat)	Dark	31.2			34.0		27.0		
Relative amount of	Light	1.25	1.14	1.07	1.04	0.96	0.89	0.0650	0.5093
Metmyoglobin	Dark	1.26	1.16	1.08	1.07	0.99	0.93		
L* values	Light	55.3	58.1	58.3	57.3	57.7	58.5	0.5090	0.5341
	Dark	55.0	57.7	57.7	57.3	57.5	57.7		
a* values	Light	23.4	21.6	19.9	18.7	16.2	15.3	1.012	0.0804
	Dark	23.7	22.5	20.8	20.0	17.9	17.1		
b* values	Light	20.1	20.3	19.4	18.7	17.7	11.4	0.7000	0.5294
	Dark	20.4	20.9	19.7	19.1	18.5	18.4		
Chroma	Light	31.1	29.9	28.0	27.0	24.3	23.4	1.116	0.4651
	Dark	31.4	30.5	28.7	27.5	25.8	24.8		
Hue	Light	40.6	43.1	44.5	45.0	47.7	49.1	1.694	0.5386
	Dark	40.7	42.7	43.9	44.0	46.2	47.4		

Table 3.22 continued

		day p	day 2	day 4	day 6	day 8	day 10		
Total microbial count	Light	3.85			7.85		8.82	0.5770	0.8195
(log_{10} (CFU)/g)	Dark	3.85			7.15		9.06		
B. thermosphacta microbial	Light	2.25			6.36		6.79	0.6510	0.9563
count (log_{10} (CFU)/g)	Dark	2.21			6.11		7.21		
Lactic acid microbial	Light	3.45			6.48		7.68	0.4170	0.7680
count (log_{10} (CFU)/g)	Dark	3.45			6.20		7.54		

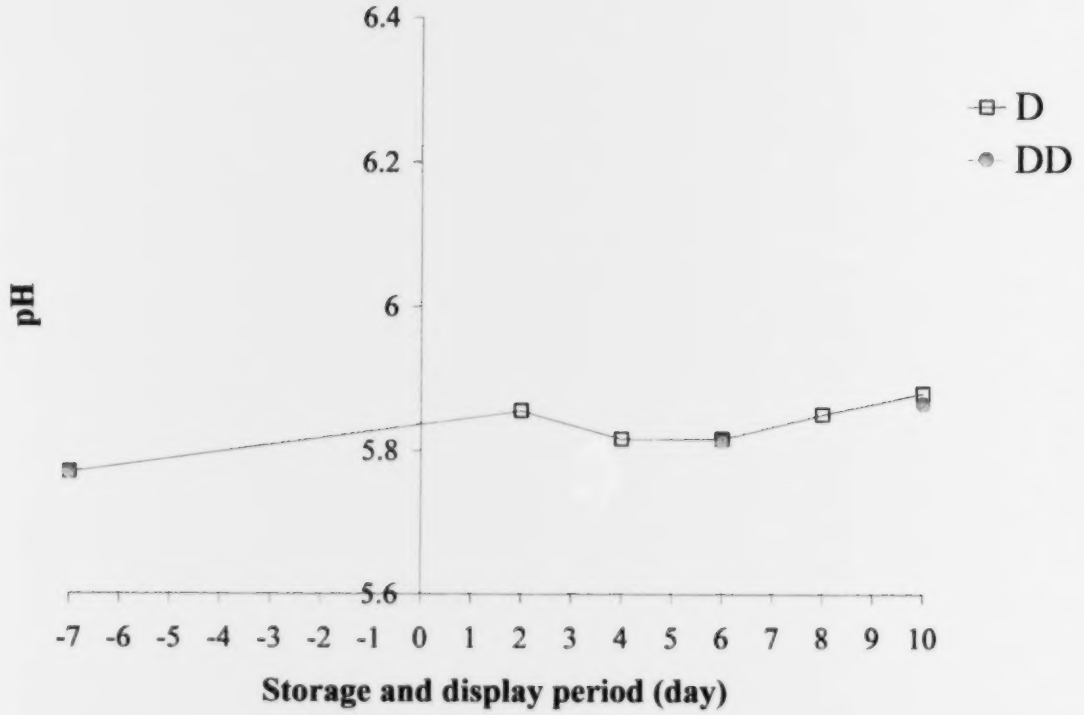


Figure 3.17: pH of formulation D fresh pork sausages that were displayed under 850 – 1100 lux illumination and formulation DD fresh sausages that were displayed in the dark at 4°C. D = 0.05% sodium erythorbate and 0.25% lemon juice powder, DD = D that was stored under dark during display period for 10 days at 4°C

4.0 CONCLUSIONS

Meat is a perishable food item, and meat spoilage is a significant concern for the meat industry. The spoilage of meat and meat products is highly correlated with shelf-life, which is the total time that a meat product remains desirable enough to be saleable. The results of my thesis research indicate that there were visible signs of deterioration in quality for both ground pork and fresh pork sausages patties over time during storage.

- The antioxidant capacity significantly correlated with colour measurement; the decrease in antioxidant activities of the patties over time contributed to meat discolouration.
- The ground pork patties discoloured faster than the fresh pork patties because the antioxidant capacity in ground pork patties was less effective than in fresh pork sausage patties.
- The a* values were significantly decreased in this study for both ground pork and fresh pork sausage patties, which means both type of patties lost their colour stability as time progressed.
- The microbial count increased more vigorously over time in ground pork patties than in fresh pork sausage patties, indicating the effect of ingredients such as salt.
- Even though ground pork and fresh pork sausage patties had different ranges of redox potential values, their redox potentials both decreased over storage time.

Non-meat ingredients that stabilize colour can improve the shelf-life of meat and meat products. One of these non-meat ingredients is ascorbic acid, a compound commonly used to delay discolouration. D-isomer of ascorbate (known as sodium erythorbate) has been widely used as food ingredient for its reducing and antioxidative traits. Its antioxidant activities include: regeneration of primary antioxidants, inactivation of prooxidant metals, and scavenging of reactive oxygen radicals (Bauernfiend and Pinkert, 1970). However, ascorbic acid is highly susceptible to oxidation, especially when catalyzed by metal ions such as Fe^{3+} (Khan and Martell, 1967). Therefore, it is logical that the addition of a chelator such as citric acid (found in lemon juice powder) could provide the greatest overall benefit for maintaining colour stability of meat products through their expected shelf-life relative to any single antioxidant class alone. In this study, however, no such synergistic interaction was observed in improving colour stability when lemon juice powder and sodium erythorbate were used at level 0.25% and 0.05% respectively.

- Only the addition of sodium erythorbate was shown to improve the colour (a*values) of fresh pork sausage by enhancing catalase activity
- The colour stability in this study was not affected by the microbial activity but rather the effect of non-meat ingredients on catalase activity because there was no significant combined effect of both sodium erythorbate and lemon juice powder on microbial activity.

The redox potential measurement made in the middle of the patty showed that sodium erythorbate lowered the redox potential in comparison to the ground pork. The reduction of redox potential might be the reason for the improved redness (a* values) in fresh pork sausage formulations with erythrobate, since the low redox environment would be expected have a better metmyoglobin reducing capacity.

5.0 FUTURE WORK

In this study, there was no synergistic interaction between lemon juice powder and sodium erythorbate at level 0.25% and 0.05%, respectively, the amount that is usually used in the industry. The concentration of the citric acid in fresh pork sausage patty which contains 0.25% lemon juice powder was only 0.066%. This amount might be too low to cause a significant effect with sodium erythorbate. Therefore, in the future, a range of levels of citric acid should be analyzed to find out the optimum concentration of citric acid that could give a significant synergistic effect with sodium erythorbate. From this concentration of citric acid, the amount of lemon juice powder can then be derived and added into the formulation. Moreover, it might prove better to add citric acid directly than to add lemon juice powder.

Further work is also needed in order to clarify the full mechanisms of how antioxidant activity, especially catalase activity, affects colour reversion on DFD fresh pork sausage because the activity of antioxidants are different at different pH as in DFD fresh pork sausage. Moreover, it would also be desirable to define the factors that could affect the *in situ* levels of reductants in meat because the reducing environment could control rates of metmyoglobin reduction to oxymyoglobin. Lastly, the effect of metmyoglobin reductase during storage on colour, microbial and redox potential should be investigated in more detail. This enzyme might have an effect on endogenous antioxidant enzymes that then affect the shelf life of fresh pork sausage.

6.0 ACKNOWLEDGEMENTS

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